ACTA RADIOLOGICA

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OFFICIAL ORGAN OF THE RADIOLOGICAL SOCIETIES OF DENMARK, FINLAND, NORWAY AND SWEDEN

THERAPY PHYSICS BIOLOGY

INDICES to Vol 12 (1973)

February April June August October December

Contents of Volume 12—THERAPY PHYSICS BIOLOGY

ACTA RADIOLOGICA

OFFICIAL ORGAN OF THE RADIOLOGICAL SOCIETIES OF DENMARK, FINLAND, NORWAY AND SWEDEN

Vol 12 Fasc 1 | THERAPY PHYSICS BIOLOGY | 1973 February

HISTOLOGIC CLASSIFICATION AND GRADING OF MALIGNANCY IN CARCINOMA OF THE LARYNX

P. Å. JAKOBSSON, C.-M. ENEROTH, D. KILLANDER, G. MOBERGER and B. MÅRTENSSON

The histologic evaluation of biopsies of possibly malignant lesions of the larynx is of importance in the choice of therapy. Epithelial changes in the larynx have been diagnosed at Radiumhemmet and the Departments of oto-laryngology and tumour pathology since 1968 by the same principles as long applied in the diagnosis of epithelial abnormalities of the cervix uteri:

Benign	Hyperplasia/metaplasia
	Papilloma (condyloma)
Possibly malignant	Dysplasia
	slight
	moderate
	severe
Malignant	Carcinoma in situ (synonyms noninvasive or preinvasive carcinoma)
	Invasive carcinoma
	squamous cell carcinoma
	highly differentiated
	moderately differentiated
	poorly differentiated
	adenocarcinoma
	anaplastic and indeterminate carcinoma

From Radiumhemmet and the Departments of Tumour Pathology and Oto-laryngology, Karolinska Sjukhuset, 104 01 Stockholm, Sweden. Submitted for publication 9 June 1972.

Table 1

Histologic grading of malignancy. Tumour cell population

	Points			
	1	2	3	4
Structure	Papillary and solid cords	Strands	Small cords and groups of cells	Marked cellular dissoc.
Differentiation	Highly keratin.	Moderately some keratin.	Poorly minim. keratin.	Poorly no keratin.
Nuclear polymorphism	Few enlarg. nuclei	Moderate number of enlarg. nuclei	Numerous irregul. enlarg. nuclei	Anapl. immature enlarg. nuclei
Mitoses	Single	Moderate number	Great number	Numerous

Table 2

Histologic grading of malignancy. Tumour host relationship

	Points			
	1	2	3	4
Mode of invasion	Well defined borderline	Cords, less marked border line	Groups of cells, no distinct border line	Diffuse growth
Stage of invasion	Possibly	Micro carc. (few cords)	Nodular into connect. tissue	Massive
Vascular invasion	None	Possibly	Few obvious	Numerous
Cellular response (plasmolymphocytic)	Marked	Moderate	Slight	None

This classification proved most helpful as a means of an improved and more distinct communication between the pathologist and the clinician.

Dysplasia represents a heterogeneous group of unknown malignancy. Present knowledge allows no prediction as to whether such lesions constitute an early stage of carcinoma or are to be regarded as reversible, epithelial proliferations related to inflammatory processes in the larynx. Each case of diagnosed dysplasia, however, requires careful control with repeat biopsies to exclude malignancy.

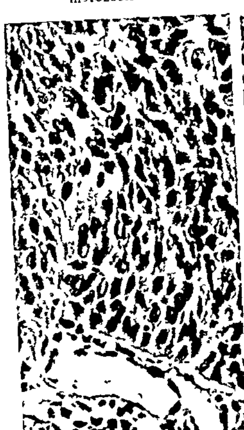


Fig 1 Squamous cell carcinoma Slight nuclear polymorphism Point value 1 Photomicrograph × 400

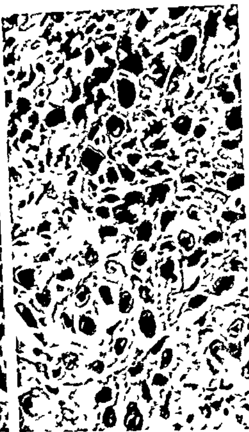


Fig 2 Squamous cell carcinoma Considerable nuclear polymorphism Point value 3 Photomicrograph × 400

Carcinoma in situ is today accepted as representing malignant neoplasia in its preinvasive stage which, according to the definitions, will progress into invasive carcinoma if untreated

Invasive carcinoma The squamous cell carcinomas of the larynx have generally been registered according to their degree of differentiation (maturation) as highly, moderately or poorly differentiated tumours This estimation of the morphology of squamous cell carcinomas has not been sufficient, neither as the basis for choice of treatment (radiation therapy, surgery) nor for a correlation to prognosis Numerous tumours exhibit a high degree of differentiation, frequently with keratinization at the surface and poor differentiation within the

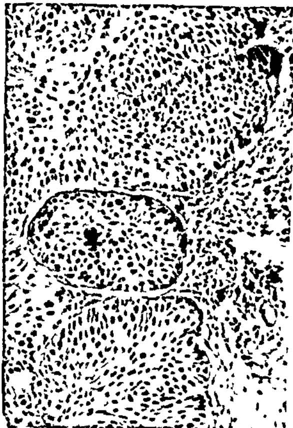


Fig 3 Squamous cell carcinoma Tumour growth in solid cords with well defined border lines Point value 1 Photomicrograph × 160



Fig 4 Squamous cell carcinoma Tumour invasion in the form of minute groups of cells Point value 3 Photomicrograph × 160

invasive parts in the deeper areas The histologic evaluation of the biopsies will therefore often be dependent on whether they are representive of the most malignant part of the tumour Estimation of the degree of differentiation of squamous cell carcinoma is also subjective and is hardly based upon well defined morphologic criteria The need for an improved morphologic grading of malignancy that could possibly serve as a more accurate measure of the biologic characteristics of the carcinomas is obvious The present investigation was performed to test a new type of histologic grading of malignancy

Material All 42 cases of invasive laryngeal carcinoma that received primary radiation therapy during 1965 to 1966 and with a five-year follow-up were investigated

Methods The histologic reclassification was always performed in the initial biopsies before the treatment The morphologic assessment was made without

Table 3

Clinical stage and histologic grade of malignancy in 42 cases of carcinoma of the larynx receiving primary radiation therapy

Histologic grade of malignancy points	Clinical stage			
	T1	T2	T3	T1 – T3
< 15	7	4	2	13
15 – 20	12	4	2	18
> 20	2	5	4	11
Total	21	13	8	42

Table 4

Results of five year follow up of 42 cases of squamous cell carcinoma of the larynx in relation to the clinical stage before irradiation

Clinical stage	Alive		Dead		Total
	Free from symptoms	With carcinoma	Of intercurrent disease	Of carcinoma	
T1	18	1	2	–	21
T2	5	3	2	3	13
T3	4	1	–	3	8
Total	27	5	4	6	42

any knowledge of the clinical stage, the treatment, or the further course of the disease

The following morphologic criteria were registered The tumour cell population was recorded on a 1 to 4 point scale (Table 1) as well as its relation to adjacent tissues (Table 2) Examples are given of tumours with different degrees of nuclear polymorphism (Figs 1, 2) and with different types of invasion (Figs 3, 4)

The criteria registered thus received points of from 1 to 4 The system permits a grading with a total of points from 8 to 32 All the cases examined fell within the range of 12 to 24 points

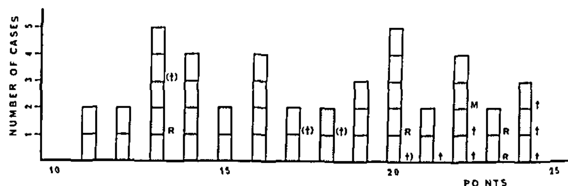


Fig. 5. Results of five-year follow-up of 42 cases of squamous cell carcinoma of the larynx in relation to clinical stage and histologic grade of malignancy as measured in a 10 to 25 point scale. Symbols: † dead of carcinoma, (†) dead of intercurrent disease, M alive with metastases, R alive with local recurrence. T1 (unfilled), T2 (dashed) and T3 (dotted bars).

Results

Clinical stage (T1, T2, T3) and the histologic grades of malignancy based on the point system appear in Table 3. It is evident that no obvious correlation exists between the clinical stage and the histologic grade of malignancy. A tendency to a relatively greater number of cases in the clinical stage T2 and T3 was evident among tumours with a high grade of malignancy (9 out of 11 cases in the group > 20 points compared to 6 out of 13 cases in the < 15 points group). The small number of cases in the material does not, however, permit a statistical evaluation.

The results from the five-year follow-up are given in Table 4 and in Fig. 5. The table illustrates a certain correlation between the clinical stage of the disease and the clinical course following radiation therapy. No less than 18 out of 21 patients with tumours stage T1 were alive without symptoms after five years whereas of 8 patients with tumours stage T3, 3 had died of the disease and one is still alive with a recurrence.

Figure 5 illustrates the correlation between the histologic grade of malignancy and the clinical course. Out of 11 cases with histologic malignancy indicated by point values exceeding 20, no less than 6 patients died from the condition, 2 have local recurrences and one patient is still alive with metastases. The clinical course of 9 of the 11 cases with a total point exceeding 20 thus indicated the presence of highly malignant tumours. Only two of these 11 patients were alive after five years with no malignant signs, both these were diagnosed, however, in clinical stage T1. Among the other 31 patients only 2 had local recurrences, one with the total point of 20 and one with 13. Five of the other patients have died from intercurrent diseases. All the other patients are alive without symptoms at five years.

Discussion

The intention of the present preliminary investigation was to test a system with a grading of a total of eight morphologic criteria each of which could presumably serve as an indicator of the degree of malignancy of the carcinomas It was considered essential to register the tumour cell population separately from the growth characteristics revealed by the relation of the tumour to adjacent tissue components The authors are aware of the fact that some of the parameters registered may be of greater importance than others for the evaluation of the degree of malignancy of the tumours, several parameters also lay parallel to each other Thus most of the carcinomas with a fairly immature pattern and a high degree of nuclear polymorphism also exhibited diffuse growth This was, however, not always present since several papillomatous, keratinizing tumours displayed evidence of considerable nuclear polymorphism and invasive growth in the form of minute cords or small groups of carcinoma cells, this rendered higher point values than would be the results of an estimation of the degree of differentiation alone This would appear to indicate the value of evaluating several morphologic criteria The end result, the total point value in each case, is likely to serve as a more accurate measure of true malignancy of the tumour than the estimation of single criteria A statistical assessment of the relative importance of the different criteria for an evaluation of the malignancy of the tumours will require a greater number of cases than now assembled

There would appear to be reason to assume that the total point value in the individual cases reflects the degree of malignancy Despite the small number of cases in this presentation the clinical course of those with high point values (6 out of 11 patients with a total point exceeding 20 have died from the carcinoma compared to none of the other 31 patients) can hardly be due to chance alone

This preliminary investigation suggests that prognosis may be possible in the initial biopsies. This, if confirmed in a larger material, may be of importance for therapeutic decisions at an early stage of the tumour development

SUMMARY

A preliminary report on the histologic grading of the malignancy of squamous cell carcinomas of the larynx is presented The clinical course of 42 cases subjected to five year follow up has given support for the validity of the grading system described

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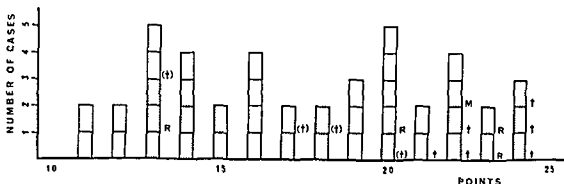


Fig. 5. Results of five year follow up of 42 cases of squamous cell carcinoma of the larynx in relation to clinical stage and histologic grade of malignancy as measured in a 10 to 25 point scale. Symbols: † dead of carcinoma, (†) dead of intercurrent disease, M alive with metastases, R alive with local recurrence. T1 (unfilled), T2 (dashed) and T3 (dotted bars).

Results

Clinical stage (T1, T2, T3) and the histologic grades of malignancy based on the point system appear in Table 3. It is evident that no obvious correlation exists between the clinical stage and the histologic grade of malignancy. A tendency to a relatively greater number of cases in the clinical stage T2 and T3 was evident among tumours with a high grade of malignancy (9 out of 11 cases in the group > 20 points compared to 6 out of 13 cases in the < 15 points group). The small number of cases in the material does not, however, permit a statistical evaluation.

The results from the five-year follow-up are given in Table 4 and in Fig. 5. The table illustrates a certain correlation between the clinical stage of the disease and the clinical course following radiation therapy. No less than 18 out of 21 patients with tumours stage T1 were alive without symptoms after five years whereas of 8 patients with tumours stage T3, 3 had died of the disease and one is still alive with a recurrence.

Figure 5 illustrates the correlation between the histologic grade of malignancy and the clinical course. Out of 11 cases with histologic malignancy indicated by point values exceeding 20, no less than 6 patients died from the condition, 2 have local recurrences and one patient is still alive with metastases. The clinical course of 9 of the 11 cases with a total point exceeding 20 thus indicated the presence of highly malignant tumours. Only two of these 11 patients were alive after five years with no malignant signs, both these were diagnosed, however, in clinical stage 1. Among the other 31 patients only 2 had local recurrences, one with the total point of 20 and one with 13. Five of the other patients have died from intercurrent diseases. All the other patients are alive without symptoms at five years.

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TREATMENT OF PAIN IN CHRONIC PANCREATITIS BY IRRADIATION

GORAN WERNER and JARL WETTERFORS

A number of factors — vascular, enzymic, infectious and traumatic, to mention only some — are involved in the development of pancreatitis The enzymic toxic effect accounts for many clinical findings Acute pancreatitis usually occurs in cases of biliary disease or chronic alcoholism It is generally diffuse and accompanied by a variety of anatomic changes and from mild to severe oedema, with or without necrosis and haemorrhage in the gland as well as fatty degeneration of the surrounding tissues

Recurrent or chronic pancreatitis may be a sequela of the acute form of the disease although it is usually a primary condition characterized by a varying degree of parenchymal fibrosis, occlusion or stenosis of the ducts, and calcification In protracted cases the gland may be transformed into a fibrous cord, with or without the presence of calculi (LAGERLOF 1967) The chronic and intermittent form often leads to invaliding pain and not infrequently to exocrine and sometimes endocrine insufficiency as well

This report deals with the treatment of chronic and painful pancreatitis with relatively large radiation doses delivered with high voltage apparatus (see the figure)

Submitted for publication 10 July 1972

RÉSUMÉ

Les auteurs présentent une note préliminaire sur la gradation histologique de la malignité des épithéliomas pavimenteux du larynx. L'évolution clinique de 42 cas suivis pendant 5 ans vient à l'appui de la validité de ce système de gradation.

Table 1

Histories and external radiation therapy in the nine cases of painful, chronic pancreatitis

Case	Sex	Age	History of			Duration of pancreatitis (yr)	Instrument	Dose (rad)	Duration of treatment (days)
			Biliary disease	Peptic ulcer	Alcoholism				
1	F	60	–	–	–	7/12	^{60}Co	5 000	56
2	M	43	–	–	+	1 × 3/12	^{60}Co	1 500	18
3	F	47	+	+	–	5	6 MV Rtg rays	2 000	33
4	M	49	–	–	–	7 × 8/12	^{60}Co	1 800	20
5	F	33	–	–	–	3	6 MV Rtg rays	1 000	16
6	M	45	–	–	+	2	^{60}Co	1 500	21
							^{60}Co	1 500	25
7	F	54	+	–	–	2 × 1/2	200 kV +Rtg rays	2 050	22
							^{60}Co	1 100	15
8	M	47	–	–	+	1	6 MV Rtg rays	2 000	33
9	M	33		+	+	2 × 5/12	200 kV Rtg rays	2 000	18

The order of the patients in the tables corresponds to the quality of the results obtained with radiation therapy beginning with the patient that responded best

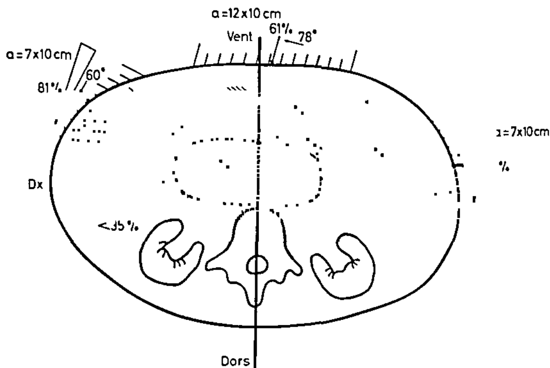
dose of 5 000 to 9 000 R was given the tissue activities of amylase, aminopeptidase and lipase were greatly reduced to levels that persisted to the end of the 12-month control period The reason for the difference between the morphologic normalization and the residual depression of the enzyme activity is still unclear The results of these and other experimental investigations may be summarised as follows

(1) The morphologic changes were always temporary, and even after a single dose of 9 000 R did not last for more than 6 months.

(2) The islands of Langerhans are less radiation sensitive than the rest of the pancreatic parenchyma, and the endocrine pancreatic function was unchanged in all the subjects examined The highest dose given was again 9 000 R (BACQ & ALEXANDER, WELLMAN et coll)

(3) The exocrine pancreatic function, however, just as the secretory volume, diminished at as low a dose as 90 R The inhibition was temporary up to about 5 000 R (CHRISHOLM & SEIBEL, RAUCH & STENSTROM)

Clinical investigations Temporary interruption of the pancreatic secretion was produced in the normal human pancreas by BRYAN & WALKER (1955) by means of conventional roentgen — 200 rad — (1 mm Cu half-value layer) and tissue doses of about 830 rad over 2 weeks to 2 240 rad over 3 weeks Moreover,



The three field technique with two wedge filters. Kilocurie therapy of 1 500 to 2 000 rad over 3 to 4 weeks as a site dose at 80 cm FSD. The dose to the kidneys was 35 per cent of that to the pancreas and that to the posterior wall of the stomach and duodenum nearly the same as the site dose.

Experimental investigations. While experimental investigations of the effect of irradiation of the pancreas in animals are fairly numerous, clinical reports remain few (FISHER & GROOT 1926, CHRISHOLM & SEIBEL 1947, RAUCH & STENSTROM 1952, BACQ & ALEXANDER 1961, WELLMAN et coll. 1966).

RAUCH & STENSTROM reported in healthy dogs that a single application of 600 R or daily doses of 200 R to a total of 800 to 1 600 R produced no histologic changes in any abdominal organs, and led to a decrease for only a short time in the volume of the pancreatic juice without any variation in the pH. The amylase, lipase and trypsin levels fell significantly 12 to 36 hours after irradiation. WELLMAN et coll. by means of electron microscopy demonstrated the effect of a single large dose of roentgen irradiation (5 000 to 9 000 R) on the fine structure of the pancreas of the dog, examined at intervals of 3 weeks to one year after the treatment. They divided the changes into 3 phases — the degenerative, the recovery (beginning at the third week) and the normalizing phase, during which the ultrastructural pattern of the cells returned to normal, 6 to 9 months after the radiation therapy. They also observed that when a large single

Table 3

Glucose tolerance in painful chronic pancreatitis Rate of elimination, K, in a semilogarithmic plot Normal range 100 K > 1

Case	1	2	3	4	5	6	7	8	9
Rate of elimination, K									
Before radiation therapy			0 66*	0 58*		0 730*		0 701*	
At 1 month follow up		1 098			1 292	0 713*	0 85*	0 827*	0 58*

* Pathologic value

patients who had had symptoms of pancreatitis for more than 7 months were treated, the length of history ranging from 7 months to 7 1/2 years with a mean of about 3 years The flow from the pancreatic duct was unrestricted in all the patients Six patients were irradiated for a recurrence after pancreatic resection Although the diagnosis of chronic pancreatitis is primarily a clinical one, it was confirmed in 6 of the 9 patients by histologic examination of the surgical specimen

Clinical examinations Before and one month after completion of the treatment the patient was submitted to the following examinations weight, peripheral blood picture, amylase in the blood and urine, pancreatic enzymes after stimulation with secretin (B Schutz, Department of internal medicine), blood glucose curve, intravenous glucose tolerance test, liver tests, electrophoresis and serum creatinine, roentgen examinations of the urinary tract and stomach and, in 5 patients, pancreatic angiography All the patients were then controlled carefully over a period ranging from 3 months to 3 years, with a mean of 2 years The pancreatic function tests were repeated 6 to 11 months after the irradiation in 3 patients (Table 2) All the patients were given constant pancreas substitution before, during and at least one month after the treatment was completed

Radiation therapy Two of the patients were given 200 kV roentgen radiation with a Thoreus filter, 4 of the patients received ^{60}Co kilocurie, and 3 patients 6 MV roentgen radiation from a linear accelerator (Varian) (Table 1) The three field technique and dose planning were always applied (Figure, Table 2) The precision of the treatment was ensured by it consistently being administered in the supine position on a fasting stomach and after anticholinergics and antiacid agents (SYLVEN et coll)

Table 2

Pancreatic secretion after stimulation with secretin expressed as a percentage of the normal values for amylase, trypsin and lipase. Lower limit for the normal value 55 per cent

Case	Steatorrhoea	Pancreatic secretion, %			
		Before radiation therapy	Follow-up period		
			1 month	6–11 mo	24–26 mo
2	+	3.1	3.9	6.9	4.0
4		38.3	26.4		
5		70	50		
6		44.6	25.2	27.2	
7		109.9	54.8	102.6	
8	++				
9		177.0	50.0		

SYLVÉN *et coll.* (1969) estimated that the tolerance dose for the mucosa in the pyloric region is about 4 300 rad ± 10 per cent, given over 5 weeks. Since, even with the best conceivable planning for high-voltage irradiation, the pyloric region falls within the 100 per cent isodose area, this level cannot be exceeded without incurring a risk of radiation gastritis and peptic ulcer.

The enzyme levels in acute pancreatitis have proved to decrease as a result of irradiation and here, too, an anti-inflammatory effect has been observed. The patients obtained relief of pain, and early introduction of the treatment sometimes banished the symptoms completely (MORTON & WIDGER 1940). A few authors have also reported the effect of irradiation on the pancreas in pancreatic fistulas and cysts as well as in chronic pancreatitis. VOLKOVA & SAROVA (1964) reported the results obtained with fairly small doses (500 R) in 56 patients with chronic, painful, recurrent pancreatitis and in 12 with pancreatic fistulas, 33 of these patients were irradiated for recurrences after operation. The symptom-free time in 26 of these 33 patients after operation was between 2 months and 2 years. After a control period of 2 years 37 patients were completely relieved of pain, 17 thought they were better and 14 derived no benefit. No harmful side effects occurred. These authors regarded the pain-alleviating effect of the treatment in pancreatic fibrosis as moderate.

Materials and Methods

Fairly large doses of high-voltage irradiation were given to 9 patients — 5 men and 4 women with an average age of 46 years. Only 2 of the patients did not have a history of biliary disease, peptic ulcer or alcoholism (Table 1). Only

after the earlier treatment Since only 2 patients had steatorrhoea, the substitution therapy could be gradually withdrawn in the other 7

Discussion

Chronic pancreatitis usually runs an irregularly remitting course and it would therefore have been an advantage to have used a control group, the total available material was however too small to allow such a division into 2 groups As the history of the disease in this material averaged as long as 3 years, the patients examined may be said in some measure to have constituted their own controls

To judge from the results in animal experiments the moderately high doses used might have produced a morphologic followed by a functional recovery of the enzyme-producing cells (RAUCH & STENSTROM, WELLMAN et coll) The pain alleviating effect would probably also have been more durable with these moderately high doses The optimal radiation dose and fractionation time for planned high voltage treatment can however only be ascertained through further clinical experiments A longer control time and enlargement of the material might also provide an answer to these questions, for the present it can be suggested only that the results are at least as good as those earlier described by VOLKOVA & SAROVA About two-thirds of the patients have been free from pain for at least two years, a fact that would appear to justify the further development of the method of radiation therapy in the treatment of painful chronic pancreatitis

Patients suitable for treatment should be chosen with due consideration given to the pancreatic secretion volume, since alleviation of pain would seem to be greatest in those in whom the exocrine function of the pancreas before the treatment is low (see Tables 1, 2) Irradiation would appear to be followed by a temporary reduction in pancreatic secretion The fact that the relief of pain is, however, not proportionally correlated with the reduction in the exocrine gland function (Table 2) suggests that the mechanism in chronic pancreatitis differs from that in the acute form of the disease Apart from the reduction in the pancreatic enzymes — amylase, trypsin and lipase — examined account must however also be taken of the general anti inflammatory and antiphlogistic effect of irradiation However since the evaluation of pain is, of course, subjective, it is difficult to explain fully the alleviating effect

Radiation therapy proved to be of value in cases of chronic recurrent or chronic fibrosing pancreatitis with pain, where there was no evidence of pancreatitic duct obstruction To avoid harmful side effects and to aim at permanent results the treatment should be given according to a plan with modern high-voltage apparatus permitting the use of suitable dosage

An incorrect diagnosis of pancreatic carcinoma resulted in the first patient receiving 5 000 rad as a site dose to the pancreas over 56 days The other 8 patients were given 1 500 to 2 000 rad over 17 to 33 days Two of the patients had a second series of treatments for recurrence of pain after the first series

Results

The most important result is that the first 6 of the 9 patients in Tables 1, 2 and 3 were completely relieved of the attacks of pain throughout a mean control period of 2 years and the alleviation of pain seemed to be greatest when the exocrine function of the pancreas before the treatment was low A temporary reduction in the exocrine pancreatic secretion was recorded According to the pancreatic enzyme determinations after stimulation with secretin in 4 of the 6 patients (Cases 4, 6, 7 and 9) given 1 500 to 2 000 rad the exocrine pancreatic function diminished to between one third and one half of that recorded before the irradiation, 2 patients (Cases 2 and 8) had initial pancreatic insufficiency and 6 out of the 9 a pathologic intravenous glucose tolerance (Tables 1, 2, 3) Case 1 was not examined with pancreatic secretion after stimulation with secretin because the method had not been evolved and Case 3 because of a prior operation (gastric resection with gastrojejunostomy) Diabetes from which one patient (Case 1) was suffering and for which she was taking drugs disappeared during the course of the treatment (5 000 rad) In the other 8 patients the endocrine pancreatic function was unaffected, as reflected in the blood sugar curve and intravenous glucose tolerance before the therapy and one month afterwards The other laboratory examinations continued to yield normal values As regards the roentgen examinations, urography — supplemented with scintigraphy — and serum creatinine tests revealed normal renal function before the treatment and one month afterwards The only serious side reaction was transient gastritis in Case 4 which, it may be noted, had a history of peptic ulceration Five of the patients, however, complained of exhaustion for some months after treatment The state of 5 patients in whom pancreatic angiography was performed had not changed significantly one month after irradiation The appearances were typical of chronic pancreatitis with stenosis of the vessels and irregular vascular lumina Three of the 6 patients whose painful attacks of pancreatitis ceased developed a dull aching pain in the right epigastrium Dyspepsia and diarrhoea diminished gradually, and sometimes disappeared completely It was usually not until a few months after treatment that an increase in weight amounting to several kilogrammes could be recorded The other 3 patients reported some subjective improvement with intervals of freedom from pain, changing after 3 to 9 months to periodic relatively severe attacks of pancreatitis, in 2 this prompted renewed irradiation, with good and, so far, permanent results 7 and 22 months

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COMBINED CHEMOTHERAPY AND RADIATION THERAPY IN SPINDLE AND GIANT CELL CARCINOMA OF THE THYROID GLAND

Report of a case

A WALLGREN and T NORIN

Anaplastic carcinomas of the thyroid of the spindle and giant cell type are almost always fatal (WOOLNER et coll 1961, SMEDAL & MEISSNER 1961, SHELINE et coll 1966, CLARK et coll 1967, BEEMER & BAKER 1970), and only seldom discovered sufficiently early as to be operable (CLARK et coll 1967) SHELINE et coll (1966) failed to observe any effect from radiation therapy in five cases with known residual disease, in fact the condition progressed during the course of treatment, as has also been our experience Extremely good response was however obtained in a case now reported of the spindle and giant cell type of thyroid carcinoma treated by combined chemotherapy and radiation therapy Twelve patients in all with inoperable or recurring spindle and giant cell carcinoma of the thyroid were treated from 1960 to 1970, but all died from the condition in the neck within a year This corresponds to the experience of other authors (SMEDAL & MEISSNER 1961, SHELINE et coll 1966, BEEMER & BAKER 1970)

Submitted for publication 22 June 1972

SUMMARY

Nine patients who had had painful pancreatitis for over seven months were treated with rather large doses of high voltage irradiation. Most became free from pain for at least two years as a result which fact appears to justify further development of this method of treatment

ZUSAMMENFASSUNG

Neun Patienten, die an einer schmerzhaften Pankreatitis mehr als 7 Monate gelitten hatten, wurden mit ziemlich hohen Dosen von Millionvolt-Bestrahlung behandelt. Die meisten waren für mindestens zwei Jahre danach schmerzfrei, was die weitere Entwicklung dieser Behandlungsmethode rechtfertigen mag.

RÉSUMÉ

Neuf malades qui avaient *une pancréatite douloureuse depuis plus de 7 mois* ont été traités par d'assez forte doses d'irradiation de haute énergie. La plupart ont été soulagés de leurs douleurs pendant au moins deux ans; ce résultat paraît justifier une expérimentation plus étendue de cette méthode de traitement.

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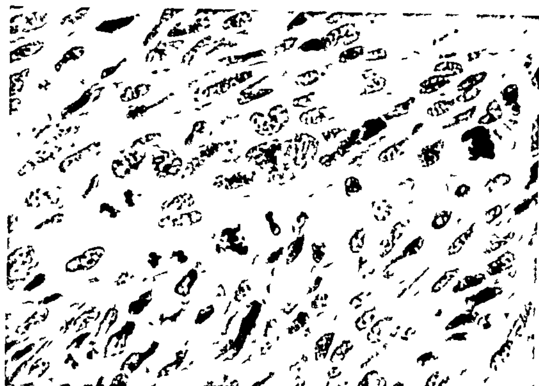
COMBINED CHEMOTHERAPY AND RADIATION THERAPY IN SPINDLE AND GIANT CELL CARCINOMA OF THE THYROID GLAND

Report of a case

A WALLGREN and T NORIN

Anaplastic carcinomas of the thyroid of the spindle and giant cell type are almost always fatal (WOOLNER et coll 1961, SMEDAL & MEISSNER 1961, SHELINE et coll 1966, CLARK et coll 1967, BEEMER & BAKER 1970), and only seldom discovered sufficiently early as to be operable (CLARK et coll 1967) SHELINE et coll (1966) failed to observe any effect from radiation therapy in five cases with known residual disease, in fact the condition progressed during the course of treatment, as has also been our experience Extremely good response was however obtained in a case now reported of the spindle and giant cell type of thyroid carcinoma treated by combined chemotherapy and radiation therapy Twelve patients in all with inoperable or recurring spindle and giant cell carcinoma of the thyroid were treated from 1960 to 1970, but all died from the condition in the neck within a year This corresponds to the experience of other authors (SMEDAL & MEISSNER 1961, SHELINE et coll 1966, BEEMER & BAKER 1970)

Submitted for publication 22 June 1972



Photomicrograph from the tumour of the thyroid. Spindle and giant cell type of carcinoma.

Case report

A woman aged 70 with a history of a nodular goitre for at least twenty years had received 4 treatments with ^{131}I for hyperthyroidism about six years previously with a total dose of 27 mCi. A month before admission she had noticed a mass in the lower aspect of the right side of the neck which had rapidly increased in size. On examination this was *found to be 5 cm in its greatest diameter and displacing the trachea to the left.* No cervical lymph nodes palpable. A radioiodine scan indicated that the thyroid gland was displaced to the left by the mass, which displayed virtually no uptake of radioiodine. Operation revealed that the growth was infiltrating the surrounding tissue including the vascular sheath; most was removed although a residue had to be left. The patient had paralysis of the right recurrent nerve postoperatively.

Microscopy disclosed a neoplasm consisting of bundles of spindle-shaped cells in a whorl-like pattern, numerous giant cells and abundant mitosis in a nodular goitre (Figure). One month later the patient was transferred for therapy. She then had a hard mass 7 cm in its greatest diameter in the right side of the neck and fixed to the larynx. External irradiation with ^{60}Co teletherapy was started through one anterior portal covering the whole neck with a daily dose of 300 rad (maximal dose). The mass after 3 000 rad had grown perceptibly. The irradiation was continued to a total maximal dose of 4 800 rad over 21 days supplemented by 500 mg 5-fluorouracil on alternate days and 200 mg cyclophosphamide intravenously every day, a total dose of 3 500 mg 5-fluorouracil and 2 800 mg cyclophosphamide was delivered. The only toxic effect produced was slight leucopenia. When

the patient was discharged one week after the completion of treatment the size of the mass was the same as on admission One month later no tumour was palpable and about five months after this the patient was in good condition and without clinical signs of recurrence Three months later and 9 months after the treatment, however, roentgenography revealed pulmonary metastases

Discussion

Radiation therapy alone appears to have failed to have had any beneficial effect in the treatment of this case with a recurrence after surgery for an anaplastic thyroid carcinoma of the spindle and giant cell type In fact the tumour increased in size during the time of irradiation which corresponds to our previous experiences with this condition as well as with that of other authors (SMEDAL & MEISSNER 1961 SHELINE et coll 1966, BEEMER & BAKER 1970) CLARK et coll (1971) reported better results by the surgical removal of as much tissue as possible followed by combined radiation therapy and chemotherapy, the latter consisted of repeated courses of actinomycin D given at periodic intervals whether recurrence or metastases were or were not present The addition of the two chemotherapeutic agents in our case seems to have been of great value Whether the effect obtained was due to chemotherapy alone or to the combination of radiation therapy and chemotherapy it is difficult to state It is possible that repeated courses of chemotherapy might have been of value in preventing or postponing the development of metastases

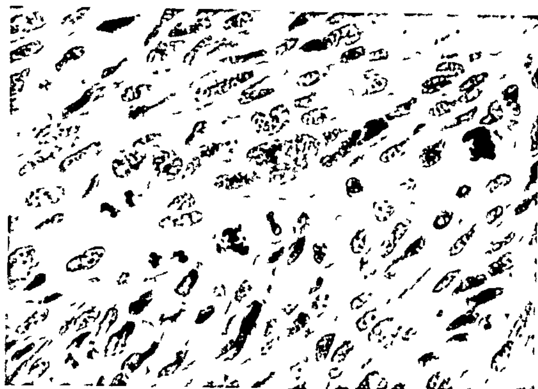
The good primary result is encouraging for further trials of this combined therapy in other cases of this highly malignant and rare disease

Acknowledgements

The authors wish to express their gratitude to Prof J Einhorn for his valuable support and aid in the preparation of this paper as well as to T Lovhagen who performed the histologic examinations

SUMMARY

A case of anaplastic carcinoma of the thyroid of the spindle and giant cell type is described A postoperative residue was treated by a combination of radiation therapy and two chemotherapeutic agents 5 fluorouracil and cyclophosphamide, in a single course and resulted in its complete clinical disappearance Further trials with this kind of combination therapy are indicated



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FIFTEEN-YEAR SURVIVORS IN HODGKIN'S DISEASE

T LANDBERG and C G AHLSTROM

A review of long term survivors in Hodgkin s disease may produce valuable information regarding the therapy and prognostic criteria Several such surveys have been published, mostly of 5 to 10 year survivals (CHAWLA et coll 1970, STRUM & RAPPAPORT 1971) It is well known, however, that recurrences of Hodgkin s disease may occur later and reports on 15 year survivors may therefore be rewarding, only a few such reports have appeared (SLAUGHTER & CRAVER 1942 BICHEL 1955, EASSON & RUSSEL 1963, MUSSHOFF & BOUTIS 1968, SMETANA 1969, PETERS 1971)

The present paper deals with 13 patients with Hodgkin s disease who survived for at least 15 years after the initial treatment Four of them died 16, 19, 20 and 21 years respectively, after the first treatment and the autopsy findings from these are included in the report

Material and Methods The 13 patients belong to a group of 98 patients with Hodgkin s disease treated during the period 1944—1956, all of them thus having the possibility of a 15 year survival on 1 January 1972 The patients are included in the communication of LANDBERG & LARSSON (1969) and the numbers

Submitted for publ cation 20 June 1972

ZUSAMMENFASSUNG

Ein Fall eines anaplastischen Karzinoms der Thyreoidea vom Spindel- und Riesenzelltyp wird beschrieben. Ein postoperativer Rest wurde mit einer Kombination von Strahlen*therapie und Chemotherapie mit zwei Substanzen, 5-Fluorouracil und Cyclophosphamid* in einem einzelnen Umgang behandelt, was zu dessen vollständigem klinischen Verschwinden führte. Weitere Versuche mit dieser Art von Kombinationstherapie sind angezeigt.

RÉSUMÉ

Description d'un cancer anaplastique de la thyroïde du type à cellules en fuseaux et géantes. Un résidu postopératoire a été traité par l'association de traitement par les radiations et de deux agents chimiothérapiques: le 5-fluorouracile et le cyclophosphamide en un seul traitement, une disparition clinique complète a été obtenue. Il est indiqué de faire d'autres essais avec ce type de traitements associés.

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Table 1

Number of survivors at different intervals 1 to 15 years after the initial treatment

		Years after the beginning of treatment														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
48 patients with localized disease	Alive	40	30	28	27	23	23	20	19	18	18	16	14	13	13	13
	Dead	8	18	20	21	25	25	28	29	30	30	32	34	35	35	35
50 patients with generalized disease	Alive	16	10	6	6	4	3	1	0	0	0	0	0	0	0	0
	Dead	34	40	44	44	46	47	49	50	50	50	50	50	50	50	50

allocated to them in the present report are the same as those of these authors Only clinically involved regions were irradiated in all 13 patients The techniques used were those described by LANDBERG (1969) Patients with advanced disease usually received chemotherapy

The original biopsy sections and the paraffin blocks were available in all instances, the blocks were recut and additional staining was carried out LANDBERG & LARSSON classified their cases according to LUKES et coll (Rye Conference 1965) The classification was confirmed in the present report except in two cases (Cases 94 and 58) in which the diagnoses were revised according to the principles laid down by LUKES at the Ann Arbor Conference 1971

Table 1 gives the numbers of survivors at different intervals after the beginning of treatment and Table 2 the clinical data of the 13 patients who survived for at least 15 years after the initial treatment

Results and Discussion

Eighty-five of the 98 patients had died within 14 years of the initial treatment The cause of death in 83 was clinically Hodgkin s disease whereas the exact cause of death was unknown in 2 patients who died after 25 and 85 months, respectively, without having presented clinical signs of recurrent disease Thirteen patients survived for at least 15 years after the initial treatment and form the basis of this report

The location of the lymphomas at the beginning of treatment is given for each of the 13 patients in figure 1 The disease was confined to a single lymph node group in 5 cases (stage I) or to two or more adjacent groups in 8 cases (stage II) In 9 cases the mediastinum was affected either as the sole site or together

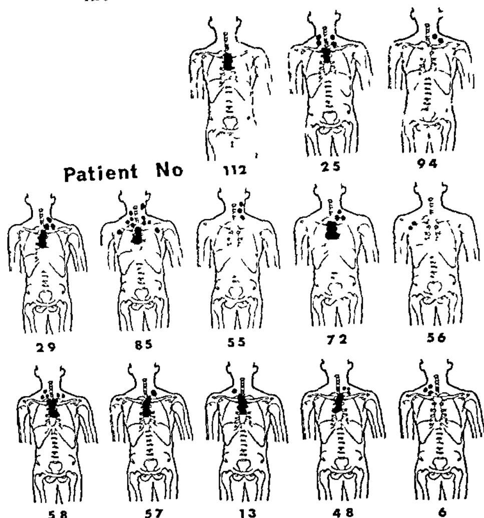


Fig 1 Manifestations at the beginning of treatment in 13 patients who survived at least 15 years after the initial treatment

with enlarged nodes in the lower neck region (8 cases) The well known fact that mediastinal Hodgkin s disease is compatible with a protracted and benign course is documented by the present material

Forty-eight of the 98 patients were considered to have localised disease (stage I or II) The 13 cases thus represent a 15 year survival rate of 27 per cent, which is lower than the age corrected survival rate of 40 per cent for patients with a

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Table 2 (*cont*)

85	55	72	56	58	57	13	48	6
M	F	M	M	M	F	F	F	M
29	30	31	33	33	34	37	38	40
Ns	Lp	Ns	Ns	Lp	Mc	Ns	Ns	Ns
7	3	54	4	36	25	1	2	12
No	–	No	No	No		–	No	No
No	No	–		No	–	–	No	No
No	No	–	–	No	–	–	No	Yes
No	No			No	No	–	No	No
18	7	15	2	58–15	55–110	15	22	5
11 400	6 100	5 200	6 600	2 300	6 200	5 900	4 600	7 000
2 600	2 000	1 200	2 800	300	1 600	1 000	1 200	1 500
		Neg	–	Pos	Neg	–	–	Neg
0	0	0	0	2	2	0	0	0
17	20	16	19	20	20	19	21	21
Alive	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead

sclerosis cellular phase This was also indicated by the finding in one of the cases of fibrous thickening of the lymph node capsule which included small collections of lymphocytes and lacunar cells

The lymph nodes in 2 patients presented typical lymphocyte predominance It is noteworthy that in one of them (Case 58) not only the supraclavicular nodes but the lymph nodes *in the mediastinum* were also affected The material of 117 cases of Hodgkin s disease published by KADIN et coll (1971) contained no patient with lymphocyte predominance and evidence of mediastinal disease

The *mediastinal* lymph nodes were also engaged in both cases of mixed cellularity The benign and protracted course of mediastinal disease seems in this material to apply not only to the nodular sclerosing type but also to other types

An impressive observation in the present material was the focal involvement of the lymph nodes with partial preservation of the sinusoidal and follicular

Table 2

Thirteen patients alive 15 years after the initial treatment Sex, age histologic type in biopsy specimen length of history, available data on constitutional symptoms, blood values and the Mantoux reaction number of prognostic forecaster index points (Westling 1965) as well as length of control and present status are given — — No information Lp — Lymphocyte predominance Ns — Nodular sclerosing type Mc = Mixed cellularity

	Case number			
	112	25	94	29
Sex	M	F	F	[illegible]
Age (years)	15	20	22	28
Histologic type in biopsy specimen	Ns	Ns	Ns	Mc
Length of history (months) up to beginning of treatment	2	36	1	2
At beginning of treatment				
Fever	No	—	No	Yes
Night sweats	—	—	—	—
Pruritus	—	—	—	
Weight loss	—	Yes	—	
ESR (mm/1 h)	63	—	14	70
White cells/μl	9 [illegible]00	10 700	7 000	[illegible] 000
Lymphocytes/μl	1 300	900	2 300	1 100
Mantoux reaction	—		—	
Number of forecaster index points (WESTLING 1965)	1	[illegible]	0	2
Follow up (years)	1[illegible]	23	17	20
At end of follow up	Alive	Alive	Alive	Alive

localized disease after 15 years given by LASSON & RUSSEL (1963) Lymphography and staging laparotomies were not carried out during the period 1944—1956 but would probably have revealed advanced disease in several of the 48 patients Such a selection would bring the 15-year survival figures in better conformity with the survival figures of the present day

The anatomic type of the disease is illustrated for the thirteen 15 year survivors in figure 2 with schematic drawings from representative sections of the lymph nodes Nine cases were classified as of the nodular sclerosing type, in 3 of them, however, the degree of sclerosis was minimal and appeared only as single bands of collagen, often extending from the lymph node capsule and incompletely surrounding cellular nodules One of these cases was originally classified as 'lymphocyte predominance However, on re-examination the nodules proved to contain numerous lacunar cells, thus revising the classification to 'nodular

Table 2 (*cont*)

85	55	72	56	58	57	13	48	6
M	F	M	M	M	F	F	F	M
29	30	31	33	33	34	37	38	40
Ns	Lp	Ns	Ns	Lp	Mc	Ns	Ns	Ns
7	3	54	4	36	25	1	2	12
No	—	No	No	No	—	—	No	No
No	No	—	—	No	—	—	No	No
No	No	—		No	—	—	No	Yes
No	No	—	—	No	No	—	No	No
18	7	15	2	58–15	55–110	15	22	5
11 400	*6 100*	*5 200*	*6 600*	*2 300*	6 200	5 900	4 600	7 000
2 600	2 000	1 200	2 800	300	1 600	1 000	1 200	1 500
		Neg	—	Pos	Neg	—	—	Neg
0	0	0	0	2	2	0	0	0
17	20	16	19	20	20	19	21	21
Alive	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead

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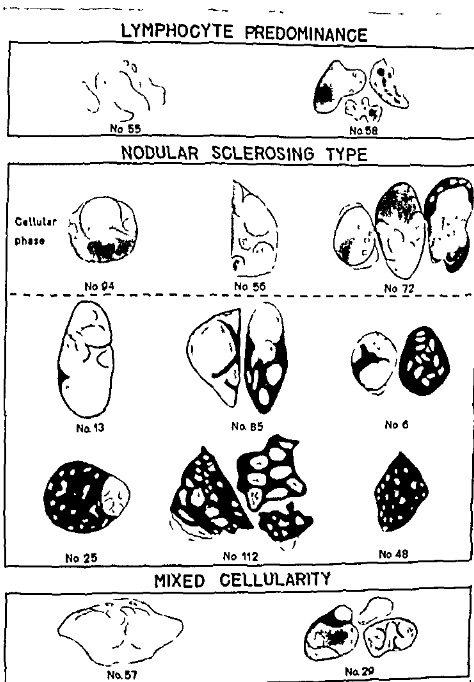


Fig 2 Schematic drawings from representative biopsy sections Black connective tissue Grey cellular proliferation of Hodgkin type White normal lymphatic tissue



Fig 3 Chest roentgenogram of Case 58 twenty years previously Expansive lesion in the superior mediastinum with lymphomas at the left hilum

pattern observed in no less than 9 of the 13 patients Focal or partial involvement of lymph nodes is considered by LUKES (1971) as evidence of recent involvement, either as an extension in the lymph node of a single region or as the initial lymph node in a new region This is valid only for 4 of the 8 patients in this material in whom the length of disease up to the beginning of treatment was less than twelve months In the other 5 patients the length before therapy was 12 months in one and 25 to 54 months in the others All specimens for biopsies were taken from regions where enlarged nodes had been observed from the beginning It appears that a partially preserved normal follicular pattern in a lymph node obtained from a region with a long history of l ... enlarge-

... The total ... of 98 patients had a sex ratio of 60 38 and for the 48 pat... clinically local... cant diff...
The age ... survivors varied between 15 and 40 years which corresponds to the experience that the survival rates are much better in young adults than in those over 50 (WESTLING 1965) The duration of disease before



Fig 4 Case 58 Cervical lymph node Hodgkin's disease of lymphocyte predominance type Hematoxylin van Gieson × 400



Fig 5 Case 58 Cervical lymph node with extensive hyalinization obtained at autopsy 20 years after the initial treatment Hematoxylin van Gieson × 20

the beginning of treatment was in 8 patients 7 months or less and in the remaining 5 patients it was 1 to 4 5 years This protracted course is of interest from two points of view First, it is consistent with the observation by PETERS & MIDDLEMISS (1958) that the longer the duration of the disease before treatment the better the 5-year survival rate, a paradoxical finding which has also been described for some other tumours and which reflects the operation of selective factors Secondly, the protracted course may support the recent hypothesis of *ORDER & HELLMAN (1972) of Hodgkin s disease being due to a chronic immune* reaction leading to the appearance of neoplastic reticulum cells

EASSON & RUSSEL reported that the survival curve after 10 years in patients with a localised lesion treated by radiation therapy approached that of the corresponding normal population, suggesting that cure had been effected Three patients in the present material are exceptions to this general rule

Case 94 Female, aged 22, who had for a month noted a lump in the left side of the neck Biopsy revealed the cellular phase of the nodular sclerosing type of Hodgkin's disease The lymphomas regressed after irradiation The patient remained without symptoms or signs for six years when she developed mediastinal lymphomas Two years later lymphomas appeared in the right side of the neck and two years after that in the left axilla All manifestations were successfully treated by irradiation Abdominal lesions and mediastinal recurrences have been recorded but the patient, who has received chemotherapy, is still alive 17 years after the initial treatment

Case 72 Male, aged 31, who had for more than four years noted lumps, slowly increasing in size, in the left side of the neck Roentgenography revealed an expansive lesion of the mediastinum and hilar lymphomas Biopsy disclosed Hodgkin's disease of the nodular sclerosing type, cellular phase The lymphomas regressed after irradiation and the patient remained free from symptoms or signs for almost 15 years until abdominal lymphomas, splenomegaly and enlarged lymph nodes in the groins developed A node from the left inguinal region disclosed Hodgkin's disease, mainly of the same type as before Irradiation but no chemotherapy was given Intestinal symptoms later arose, the general condition deteriorated and the patient died 16 years after the initial treatment At autopsy the retroperitoneal lymph nodes and the spleen displayed diffuse fibrosis associated with depletion of lymphocytes Numerous ulcers were evident in the intestinal mucosa, some of them covered with a granulation tissue containing single Reed Sternberg giant cells In addition, deposition of amyloid had occurred in the spleen, lymph nodes and in the intestinal walls themselves, as well as in the kidneys

Case 6 Male, aged 40, who had for one year noted a lump in the right side of the neck Biopsy revealed Hodgkin's disease of the nodular sclerosing type Irradiation produced regression One year later the right infraclavicular region became involved and was treated Lymphomas in the right axilla were irradiated a year later The patient remained well for another 7 [illegible]
in good co[illegible]
the disease [illegible]
numerous [illegible] to [illegible]-sized nodules in the lungs and a circumscribed mass, size of an orange, at the left [illegible]
were present in t[illegible]
yellowish nodule[illegible]

Two of the thirteen 15 year survivors thus succumbed to their disease after 16 and 21 years, respectively, and one is living with persistent symptoms and signs Two of the remaining 10 patients have died from other diseases, autopsy at 19 and 20 years, respectively, after the first treatment revealed no evidence of Hodgkin's disease

Case 13 Female, aged 37, who for a month had observed a [illegible]
neck [illegible]
node f[illegible]
band c[illegible]
[illegible] small follicles was found Irradiation

was administered The patient kept well for 13 years when she developed brownish tanning of the skin and was found to have Addison's disease Substitution therapy enabled her to live another 6 years no overt evidence of Hodgkin's disease Autopsy 19 years after the initial irradiation demonstrated extreme atrophy of the adrenal glands with only a few cortical cells around remnants of the medulla Death was due to purulent bronchitis no signs of Hodgkin's disease

Case 58 Male aged 33 with a lump in the right side of the neck for three years and multiple nodules on the left side for two years Roentgenography demonstrated an expansive lesion of the superior mediastinum and lymphomas in the left hilum (Fig 3) Biopsy of a cervical lymph node demonstrated Hodgkin's disease *of nodular type of lymphocyte predominance* (Fig 4) Small mostly marginal areas with preserved follicles were evident in all nodes The patient remained well for about 20 years after irradiation until he developed myocardial infarction Roentgenography revealed pleural abnormalities due to irradiation *but no other abnormality The patient died suddenly at home a few months later* Autopsy demonstrated that the mediastinum was transformed to dense fibrous tissue adhering to the sternum and extending to the pleura along the vertebral column the pericardial and pleural cavities were obliterated The bone marrow in the dorsal region of the spinal column was pale The heart had myocardial scarring with frank sclerosis of the coronary arteries with the left coronary artery occluded Microscopy of the cervical axillary mediastinal retroperitoneal mesenteric and inguinal lymph nodes failed to reveal any evidence of Hodgkin's disease Some of the cervical nodes were partly hyalinized probably representing scars of earlier Hodgkin's disease (Fig 5) Signs of activity were absent The spleen was normal Anteromedial parts of the lungs were slightly fibrosed

Hodgkin's disease in these last 2 patients seems to have been completely eradicated Whether the word cured may also be applied to the other 8 patients who are living without signs of active disease is open to question One of these (Case 48) has had three *spreads during the first 6* years after the initial treatment so that it may be more appropriate to speak of a long remission in spite of the fact that 15 years have passed since the last spread without any further signs of disease The other 7 patients have no relapses since the first treatment so that justification appears to exist for considering the disease eradicated and cured

It is impossible to assign the good therapeutic effect and the favourable course in the present material of 15-year survivors to a single factor An early stage of disease at the initial treatment and patients aged below 50 are certainly of primary importance The grade of the condition, i e its histologic type, seems to be significant but not decisive A partially preserved follicular structure in lymph nodes affected by the disease for many years may indicate its slow progress and benign course No influence of sex could be established in this material of long survival When the patients were grouped into different categories of prognostic forecaster index points *according to* WESTLING *(1965)*, none had 3 or more points, and 9 of the patients belonged to the prognostically favourable group with 0 or 1 point

Judged retrospectively the absorbed doses had been adequate in 11 of the patients whereas 2 patients (Cases 25 and 13) had received smaller doses. Furthermore, only those lymph node regions apparently involved had been irradiated. This appears to emphasize that the prognosis in Hodgkin's disease is due not only to readily definable but also to factors included in the term 'host resistance'.

SUMMARY

Thirteen out of 48 patients with localized Hodgkin's disease survived for at least fifteen years after the initial treatment with involved field radiation therapy. The survivors were mainly young and usually with a nodular pattern in the first biopsies. Post mortem examination in 4 patients who lived for more than fifteen years revealed no signs of the disease in 2; in the other 2 patients the disease persisted in spite of the long survival.

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Dreizehn von 48 Patienten mit lokalisierter Hodgkinscher Erkrankung überlebten zumindest 15 Jahre nach der initialen Behandlung mit einer umschlossenen Feld-Strahlentherapie. Die Überlebenden waren vorwiegend jung und gewöhnlich lag bei den ersten Biopsien ein noduläres Verhalten vor. Postmortem-Untersuchungen von 4 Patienten, die mehr als 15 Jahre gelebt hatten, wiesen bei zwei der Patienten keine Zeichen der Erkrankung auf; bei zwei anderen Patienten waren solche noch vorhanden.

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SPLIT-COURSE RADIATION THERAPY OF MEDIASTINAL HODGKIN'S DISEASE

TSD and CRE concepts

T LANDBERG, K LIDEN and H FORSLO

The radiation sensitivity of Hodgkin's disease tissue may vary from one patient to another, some having been reported (CHAWLA et coll 1970, STRUM & RAPPAPORT 1971) to stay well for long after having received only relatively small absorbed doses of radiation KAPLAN (1966) pooled data from the literature on the response to radiation therapy in Hodgkin's disease and presented a curve that indicated that the frequency of recurrence per field treated diminished with increasing dose and reached 5 per cent at the 4 000 rad level

Unfortunately it is not possible to recognise at present the patients who will do well after a relatively small absorbed dose Several factors may be of importance, e g cellular kinetics and oxygenation However, the idea has been proposed (KAPLAN 1966, JOHNSON 1971) that in the treatment of Hodgkin s disease the time factor may not be critical providing the total absorbed dose be adequate

Submitted or publication 11 July 1972

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Table 1

Histologic type in initial biopsy, type of radiation used, number of fractions, elapsed time, calculated absorbed dose in the centre of the target volume, $TSD_{^{60}Co}$ and $CRE_{^{60}Co}$ for 19 patients treated with split course radiation therapy for mediastinal Hodgkin's disease Lp = lymphocyte predominance, Ns = nodular sclerosing type, Mc = mixed cellularity

Case No	Histologic type of biopsy specimen	Type of radiation	Number of Fractions N	Number of Days t	Calculated absorbed dose (rad) in the centre of target volume: assuming water-equivalent tissue	Calculated absorbed dose (rad) in the centre of target volume: corrected for heterogeneity and normalized to ^{60}Co radiation quality	$TSD_{^{60}Co}$ ret	$CRE_{^{60}Co}$ reu
Without recurrence								
29	Mc	170 kV roentgen	32	89	4 100	5 310	2 310	1 450
47	Lp	"	36	82	3 600	4 660	1 970	1 220
58	Lp		36	89	4 400	5 690	2 410	1 520
72	Ns	"	36	82	3 900	5 050	2 140	1 310
74	Ns		30	68	5 500	7 120	3 150	2 000
85	Ns	"	32	71	3 700	4 790	2 090	1 320
94	Ns	^{60}Co	30	67	5 500	6 050	2 680	1 710
112	Ns	170 kV roentgen	28	61	4 200	5 430	2 440	1 570
114	Mc	200 kV roentgen	19	60	3 600	4 660	2 300	1 480
128	Lp	^{60}Co	33	74	4 400	4 800	2 070	1 310
158	Mc	170 kV roentgen	20	56	3 400	4 400	2 140	1 390
With recurrence								
6	Ns	170 kV roentgen	33	83	3 500	4 530	1 960	1 230
11	Lp		32	131	3 900	5 050	2 200	1 280
38	Ns	"	32	77	3 400	4 400	1 920	1 200
40	Ns		30	75	3 400	4 400	1 950	1 230
50	Mc		32	80	3 100	4 010	1 750	1 100
95	Ns		28	61	3 000	3 880	1 740	1 120
139	Mc		32	75	3 500	4 530	1 970	1 250
150	Ns	"	24	66	4 300	5 560	2 590	1 660

ELLIS (1969) postulated that the radiation tolerance of certain normal connective tissues may be expressed for different number of fractions and different lengths of treatment periods as a single value, the Nominal Standard Dose (NSD). This figure may be useful in different fractionation schedules in the calculation of the largest absorbed dose that can be given without undue risk of connective tissue necrosis. The NSD concept is not a priori applicable



Fig 1 Patients with and those without recurrence after split course radiation therapy for mediastinal Hodgkin's disease distributed among different intervals of total corrected absorbed dose in the centre of the target volume ○ = recurrence ● = no recurrence

to connective tissue reactions other than tolerance or to radiation reactions in tissues other than connective tissue For tumour cells, which under normal treatment conditions, were assumed to be subjected only to internal recovery ELLIS (1969) defined the concept of Tumour Standard Dose (TSD) ELLIS investigated the data on Hodgkin's disease reported by SCOTT (1961) and by FRIEDMAN et coll (1967) and concluded that the application of the TSD concept seemed meaningful in Hodgkin's disease

KIRK et coll (1971) agreed with the derivation of the NSD concept but suggested that the Ellis equation may be applied to any radiation effect on normal connective tissue up to and including tolerance, they introduced the concept of Cumulative Radiation Effect (CRE) Its calculation includes the length and position of any interval in the treatment

Since the true nature of Hodgkin's disease is not definitely settled—whether neoplastic or reactive—it is not possible to say which of the two concepts, TSD or CRE, may be relevant

The results of split course radiation therapy of mediastinal Hodgkin's disease have been reported in a previous paper (LANDBERG & FORSLO 1970) The material consisted of 19 patients followed up for at least three years after irradiation of the mediastinum, signs of a local recurrence appeared in 8 of the patients The presence or absence of recurrence tended to vary with the size of the retrospectively calculated absorbed dose in the centre of the target volume All patients but 2 had been treated with 170 to 200 kV roentgen rays The treatments had been given in 32 fractions (median) in 75 days (median) split course, and it seemed that with this fractionation the total absorbed dose in the centre of the target volume should not be below 3 600 rad to be effective Phantom investigations suggested that owing to tissue heterogeneity the absorbed dose in the target volume was 1 1 times that calculated from isodose charts valid for a homogeneous material

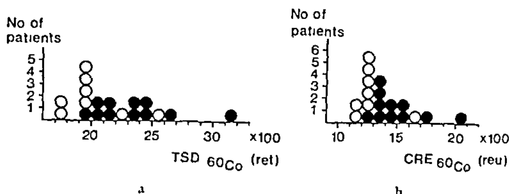


Fig 2 Patients with and those without recurrence after split course radiation therapy for mediastinal Hodgkin s disease distributed among different intervals of a) TSD_{60Co} and b) CRE_{60Co} ○ = recurrence, ● = no recurrence

The purpose of the present investigation was to express the calculated absorbed doses in the above-mentioned material in different ways This was to determine whether any particular level could distinguish between patients with and those without a recurrence, and if so, to compare the given split-course treatments of Hodgkin's disease with published results of treatment in a single series

Material and Methods Details about the clinical material and the methods used to calculate the absorbed dose in the centre of the target volume have already been published (LANDBERG & FORSLO 1970) The following equations were used in calculating the *TSD* (ELLIS *1969*) *(1)* and the *CRE* (KIRK et coll 1971) (2)

(1) $TSD = D \cdot N^{-0.24}$

(2) $R_F = D \cdot N^{-0.24} \cdot t^{-0.11}$

TSD = Tumour Standard Dose (ret)

R_F = Cumulative Radiation Effect (CRE) (reu)

D = *Total absorbed dose (rad)*

N = Number of fractions

t = Total time (days)

The data from the ^{60}Co treatments were analyzed directly with eqs (1) and (2) Those from the treatment with 170 to 200 kV roentgen rays were first standardized to ^{60}Co radiation quality by multiplication by the factor $\frac{1}{RBE_{60Co}}$ and then analyzed RBE_{60Co} chosen was 0 85

Table 2

Total corrected absorbed dose in the centre of the target volume divided by the number of fractions and the total number of days for 19 patients treated with split-course radiation therapy for mediastinal Hodgkin's disease

	Eleven patients without a recurrence			Eight patients with a recurrence		
	Range	Median	Mean	Range	Median	Mean
Total corrected absorbed dose (rad)/ Number of fractions	130-245	165	180	125-230	140	150
Total corrected absorbed dose (rad)/ Number of days	57-105	67	74	39-84	58	59

Results and Discussion

Table 1 gives the histologic type in the initial biopsy specimen, and data on type of radiation and fractionation Calculated absorbed dose in the centre of the target volume for each patient is also given, assuming that the volume treated was comparable to water equivalent tissue from the point of view of radiation absorption and then corrected for tissue heterogeneity and normalized to ^{60}Co radiation The $TSD_{^{60}Co}$ and $CRE_{^{60}Co}$ are also included in the table

The number of patients with and without a recurrence distributed among different intervals of corrected absorbed dose (rad), $TSD_{^{60}Co}$ and $CRE_{^{60}Co}$, respectively, are collected in Figs 1 and 2

The quotients between the total corrected absorbed dose (*rad*) *and number* of fractions and the total number of days, respectively, as *range*, *median and* mean for patients without as *well* as *for those* with a recurrence appear in Table 2 The *patients without* and with a recurrence seem to diverge at 4 600 rad (Table 1, Fig 1) *at* $TSD_{^{60}Co}$ *2 000* ret (Table 1, Fig 2a) and at $CRE_{^{60}Co}$ 1 300 reu (Table 1, Fig 2b)

When the *whole corrected* absorbed dose for each patient was divided by the number of fractions and the total number of days, while the range, median and mean for patients without a *recurrence were* compared with the corresponding figures for patients with a *recurrence (Table 2)*, the former presented uniformly larger values

The separation between patients without and those with a recurrence is relatively well marked *at the levels* mentioned, suggesting that the variation in radiation sensitivity in the material investigated was unimportant Table 1 indicates that most patients *had* either lymphocyte predominance or a nodular

sclerosing type of Hodgkin's disease, both being types usually running a slow clinical course Further, the lesions in these types probably fail to be impaired by oxygenation due to necrosis The relative distribution of the histologic types among patients without a recurrence when compared with those with a recurrence fails to support any assumption of a different radiation sensitivity in the two groups due to the types

The current literature contains only few reports on the results of split-course radiation therapy in Hodgkin's disease SCANLON (1963) published some cases KAPLAN (1966) concluded that an absorbed dose of 4 000 rad over 4 weeks should be considered the 'tumoricidal' dose in Hodgkin's disease Assuming 5 treatments a week, this means 4 000 rad in 20 fractions over 26 (25 to 27) days, the TSD then being 1 950 ret and the CRL 1 360 reu

The patients in the present material, having been treated in median 32 fractions over median 75 days, required a larger total absorbed dose than that recommended by KAPLAN One explanation is that a time factor may be of some importance Another explanation may be that the size of the absorbed dose at each fraction was smaller in the present material than that suggested by KAPLAN Each explanation may be valid, this being supported by the fact that both the critical $TSD_{^{60}Co}$ and $CRL_{^{60}Co}$ values in the present investigation agree reasonably well with the corresponding figures obtained from the Kaplan data

SUMMARY

Nineteen patients who had received radiation therapy for mediastinal Hodgkin s disease appeared to have a well marked dose level that separated those without and those with a recurrence Relatively small absorbed doses administered at each fraction or treatment over a long period of time may necessitate a larger total absorbed dose

ZUSAMMENFASSUNG

Neunzehn Patienten die wegen einer Hodgkin'schen Erkrankung Strahlentherapie ausgesetzt worden waren schienen eine gut abgegrenzte Dosishohe zu haben die solche Patienten ohne von denen mit einem Recidiv voneinander trennte Relativ geringe absorbierte Dosen bei jeder Fraktion verabfolgt oder Behandlung uber einen langen Zeitraum mogen eine hohere gesamtabsorbierte Dosis notwendig machen

RÉSUMÉ

Sur dix neuf malades traités par les radiations pour maladie de Hodgkin médiastinale il semble y avoir un niveau de dose bien défini qui sépare ceux qui ont présenté des récidives de ceux qui n en ont pas eu Les doses absorbées relativement petites administrées à chaque fraction ou le traitement étalé sur une longue période de temps peuvent nécessiter une dose totale absorbée plus importante

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The current literature contains only few reports on the results of split-course radiation therapy in Hodgkin's disease. SCANLON (1963) published some cases. KAPLAN (1966) concluded that an absorbed dose of 4 000 rad over 4 weeks should be considered the 'tumoricidal' dose in Hodgkin's disease. Assuming 5 treatments a week, this means 4 000 rad in 20 fractions over 26 (25 to 27) days, the TSD then being 1 950 ret and the CRL 1 360 reu.

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SUMMARY

Nineteen patients who had received radiation therapy for mediastinal Hodgkin's disease appeared to have a well marked dose level that separated those without and those with a recurrence. Relatively small absorbed doses administered at each fraction or treatment over a long period of time may necessitate a larger total absorbed dose.

ZUSAMMENFASSUNG

Neunzehn Patienten, die wegen einer Hodgkin'schen Erkrankung Strahlentherapie ausgesetzt worden waren, schienen eine gut abgegrenzte *Dosishöhe zu haben*, die *solche* Patienten ohne von denen mit einem Recidiv voneinander trennte. Relativ geringe absorbierte Dosen ... höhere

RÉSUMÉ

Sur dix neuf malades traités par les radiations pour maladie de Hodgkin médiastinale il semble y avoir un niveau de dose bien défini qui sépare ceux qui ont présenté des récidives de ceux qui n'en ont pas eu. Les doses absorbées relativement petites administrées à chaque fraction ou le traitement *étalé sur une longue période de temps* peuvent nécessiter une dose totale absorbée plus importante.



Fig 1 A lateral tangential chest wall irradiation set up showing the placement of the LiF teflon dosimeters

ficiently thin configurations, it was possible to experimentally investigate the surface effects of oblique incidence and examine the absorbed dose distribution within the superficial layers Some preliminary data of measurements made on patients and tissue equivalent phantoms were reported by us previously (MANSFIELD & SUNTHARALINGAM 1971) A discussion of some of the physical aspects of surface effects of high energy roentgen rays at oblique incidence has also appeared in the literature (JACKSON 1971, ORTON & SIEBERT 1971) In the present report, we are summarizing our experimental measurements on phantoms and patients

Methods and Materials For the measurement of surface doses and build up of dose with depth, at oblique incidence, a hemispherical plastic cup of radius 7 cm was made from a 0 5 mm thick sheet of Buterate plastic by a moulding technique Holes were drilled at appropriate angles to accommodate a stack of ten thin detectors The cup was filled with tissue equivalent bolus material

The radiation detectors used in this investigation were thermoluminescent

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DOSE DISTRIBUTION FOR COBALT 60 TANGENTIAL IRRADIATION OF THE BREAST AND CHEST WALL

C M MANSFIELD and N SUNTHARALINGAM

In the radiation treatment of patients with carcinoma of the breast it is often desirable to include the surface and superficial layers of the skin in the total volume of tissue to be irradiated Also, an attempt is made to obtain a uniformity of absorbed dose throughout the treatment volume BUSCH & JOHNS (1962), have reported on the surface effects of cobalt-60 gamma radiation at oblique incidence from phantom measurements using film dosimetry techniques They showed that the above mentioned criteria for tangential breast treatment could be reasonably satisfied only with the use of bolus during treatment The extent of the use of bolus is to be determined by the individual tolerance of skin reactions

Skin reactions following cobalt-60 treatments of the breast and chest wall areas, when irradiated with full or partial bolus, suggested that factors in addition to individual tolerance were operative Therefore an examination of the technique of irradiation with tangential opposed fields, with and without bolus, was undertaken With the availability of thermoluminescent detectors, in suf-

This investigation was supported by N I H grant No PR72 and was presented in part at the European Congress of Radiology Amsterdam June 1971 Submitted for publication 29 March 1972

Table

Tangential breast irradiations Measurements on Alderson phantom

Cobalt 60 80 cm SSD	Surface dose (per cent of tumor dose)	
	Breast	Chest wall
Opposed fields with breast applicator and with bolus 20 cm × 20 cm blocked to 20 cm × 10 cm)	100	100
Opposed fields with breast applicator but without bolus (20 cm × 20 cm blocked to 20 cm × 10 cm)	78–86	80–85
Opposed fields without breast applicator and without bolus (20 cm × 10 cm)	55–60	58–62

Results

The measurements of the build up of dose as a function of depth and the angle of incidence of the radiation beam are summarized in figure 2 These measurements were made with the hemispherical tissue-equivalent phantom arrangement, for a 15 cm × 15 cm field size at a source phantom distance of 80 cm It is seen that the surface dose for normal incidence (90°) is about 40 % of dose maximum but it can be as high as 65 % of dose maximum when the beam is tangential (0°) The dependence on the angle of incidence of the depth at which the dose reaches a maximum is also seen For tangential incidence the maximum is reached at a depth of 0 75 mm in LiF-teflon, or about 1 5 mm in tissue

While variations in oblique incidence can affect the magnitude of the surface dose, the presence of the special breast treatment cone in close proximity to the surface results in electron contamination of the photon beam and further increases the surface dose The measured surface doses, expressed as a percentage of the given tumor dose, for phantom irradiations using three different treatment techniques, are given in the Table It is quite evident that the surface dose depends on the particular treatment technique chosen and can be as high as 75 to 85 % of the tumor dose, when no bolus is used

The surface doses and the doses at points at depth within the irradiated volume measured on the Alderson phantom, for a parallel opposed tangential chest wall irradiation without bolus are given in figure 3 The calculated isodose lines are shown by the broken lines The measured doses at any one level are in close agreement, within ± 2 %, and also agree within ± 5 % of the calculated doses. The measurements also confirmed the non uniformity of dose about 30 %) within the irradiated volume when no bolus was used The

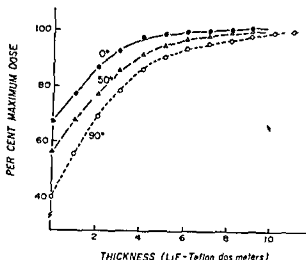


Fig. 2. Build up of depth dose as a function of angle of incidence, for cobalt 60 gamma radiation

LiF-teflon discs, 0.13 mm thick and 13 mm in diameter. A batch of dosimeters with a uniform sensitivity of ± 2 % was selected from a large group of dosimeters. Before re-use these dosimeters were annealed for five hours at 300° C and 24 hours at 80° C and then recalibrated. Surface doses on patients treated with opposing tangential fields were also measured using the LiF-teflon discs.

For the measurement of doses at points at depth within the irradiated volume, the trunk portion of the Alderson tissue-equivalent phantom was used in a simulated patient set-up. Lithium fluoride (TLD-100) powder was used as the dosimetric material. The powder was placed inside small gelatin capsules and positioned within holes appropriately spaced within the phantom. The TLD powder was annealed for 1 hour at 400° C and 24 hours at 80° C before each use and a new dose response calibration curve obtained. Surface doses on the phantom were measured using the LiF-teflon discs.

In Fig. 1 is shown a typical set-up of a patient for a lateral tangential chest wall irradiation on a Picker-C3000 Cobalt-60 machine. A special breast treatment cone is used for tangential fields and the plexiglass end plate is close to the patient's skin. The LiF-teflon disc dosimeters have been placed on the patient's skin along a line in the central plane of the radiation beam. To complete the opposing field treatment, the radiation source is rotated to the position of the medial tangential field without altering the position of the TLD dosimeters. When the use of bolus was investigated, cloth bags containing tissue-equivalent bolus were positioned on the patient's skin and placed over the TLD dosimeters.

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Fig 3

Fig 4

Fig 5

Fig 3 Measured doses, in rad for a phantom chest irradiation, without bolus using tangential opposed fields to deliver 270 rad to the calculated 270 line The calculated isodose lines are shown by the dashed lines

Fig 4 Measured skin doses in rad on a patient treated to 180 rad to point P with tangential opposed breast fields, without bolus Also shown are the calculated isodose lines

Fig 5 Measured skin doses in rad on the same patient treated with the same fields as in Fig 4, but with bolus Also shown are the calculated isodose lines

measured surface doses are about 50 to 70 % of the total delivered tumor dose of 270 rad from the two fields Again, it should be pointed out that the variation of dose along the irradiated curved surface is a function of the entrance dose, the angle of oblique incidence, the degree of electron contamination and the exit dose

The measured skin doses on a patient treated with parallel opposed tangential breast fields, using the breast applicator but without bolus, are shown in Fig 4 Also shown are the calculated isodose lines in the central plane of the treatment fields The total dose delivered by both fields to point P was 180 rad The surface doses measured about 70 to 80 % of the calculated dose to point P The skin doses measured on the same patient, when treated with the identical

tangential fileds, but with bolus, are shown in Fig 5 In this situation, the skin doses measured about 10 % higher than the calculated tumor dose of 180 rad This slight discrepancy was due to insufficient bolus used to compensate for the lack of tissue Although the lower and middle portions of the breast are covered with bolus, the bolus material should adequately fill the space between the upper portion of the breast and the plexiglass plate of the breast cone

Discussion

From physical considerations of the interaction of cobalt 60 gamma radiation, at an air tissue interface, for varying oblique incidence, it has been shown that it is possible to calculate the surface doses and the depth of dose maximum (JACKSON 1971) Our measurements confirm the above theoretical computations As a result of a large series of phantom and patient measurements it appears that, in the absence of bolus, skin doses can be as high as 70 to 80 % of the tumor dose This is due to the surface curvature and oblique incidence and also the extent of electron contamination

Oblique indicence alone shows an increase in skin doses only for beam angulations confined to near tangential incidence and even then the skin dose is increased only to about 65 % of dose maximum However, it has been common practice for some radiation therapists to consider the increase in skin doses and accompanying doses to the superficial layers, due to oblique incidence, to be therapeutically adequate and have made a case for not using bolus and thereby avoiding severe skin reactions It should be pointed out that under these circumstances, one is also accepting a non uniformity of dose as high as 35 % within the total treatment volume With a desire to achieve better uniformity of dose and also to deliver sufficient doses to the skin and underlying superficial layers, some therapists have chosen to use bolus The number of treatments with bolus and the termination of the use of bolus are determined by the individual therapists based on the skin reaction With our clinical experience during the past twelve months, during which this investigation was undertaken, we have arrived at two possible irradiation techniques using full bolus In one case a total tumor dose to midline of 180 rad is delivered by treating both fields on the same day The other method is to treat one field per day and deliver an incident dose of 300 rad The midline dose in this case will depend on field separation The detailed analysis of this clinical data will be reported later

SUMMARY

The surface dose and the dose to underlying superficial layers for cobalt 60 tangential irradiation of the breast and chest wall areas have been measured LiF teflon disc dosimeters were used Oblique incidence surface curvature and the presence of special breast applicators influenced the dose distributions

ZUSAMMENFASSUNG

Die Oberflächendosis und die Dosis der darunterliegenden oberflächlichen Schichten wurden für die tangentiale Cobalt 60 Bestrahlung der Brust und der Brustkorb Regionen gemessen Es wurden LiF Teflon Scheiben Dosimeter verwendet Der Grand des Einfalls winkels die Oberflächenkurvatur und das Vorhandensein spezieller Brustapplikatoren beeinflussten die Dosisverteilungen

RÉSUMÉ

Les auteurs ont mesure la dose en surface et la dose dans les couches sous jacentes à la surface pour l irradiation tangentielle par le cobalt 60 du sein et de la paroi thoracique Ils ont utilise des dosimetres disques LiF teflon Une incidence oblique la courbure de la surface et la presence d applicateurs speciaux pour le sein influent sur les destributions de doses

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PROGNOSTIC SIGNIFICANCE OF URETERAL OBSTRUCTION IN CARCINOMA OF THE CERVIX UTERI

ANTONIO BOSCH, ZENAIDA FRIAS and GLADYS C DE VALDA

It is generally accepted that ureteral obstruction is one of the factors that influence prognosis in invasive carcinoma of the cervix uteri, but the true incidence of this alteration, and the correlation of prognosis to the degree of ureteral obstruction, whether unilateral or bilateral involvement, and its implication in the different stages of the disease have been seldom analyzed in large unselected series of patients The present investigation was carried out to determine the influence of these factors on prognosis

Material

The investigation includes 1 086 patients with invasive carcinoma of the cervix uteri seen at the I Gonzalez Martinez Oncologic Hospital from January 1956 to December 1963 Pretreatment intravenous urography was performed in 990

Submitted for publication 15 February 1972

Table 1

Pretreatment urography

Stage	No. of cases	Not examined	Examined	Normal	Abnormal
I	124	10	114 (92 %)	109	5 (4 %)
IIA	186	10	176 (95 %)	169	7 (4 %)
IIB	205	9	196 (96 %)	184	12 (6 %)
III	444	37	407 (92 %)	346	61 (15 %)
IV	127	30	97 (76 %)	39	58 (60 %)
Total	1086	96	990 (91 %)	847	143 (14 %)

Table 2

Pretreatment urographic findings by stage

Findings	Stage					Total
	I	IIA	IIB	III	IV	
Unilateral						
Hydronephrosis						
Mild	2	5	4	8	3	22
Moderate	2	1	1	20	10	34
Severe	–	–	1	3	1	5
No function	–	1	3	16	25	45
Bilateral						
Hydronephrosis						
Mild	1	–	1	1	–	3
Moderate	–	–	1	2	3	6
Severe	–	–	–	1	4	5
No function	–	–	–	5	4	9
No function on one side, hydronephrosis on the other						
Mild	–	–	1		3	4
Moderate		–	–	4	3	7
Severe	–	–	–	1	2	3
Total	5	7	12	61	68	143

patients. Urography was not done in 96 cases, either because the patient was known to be allergic to contrast media, or the films from the initial examination were not satisfactory for diagnosis but for reasons unknown no repeat exami-

Table 3

Five year survival in patients with normal versus abnormal urography

Stage	Normal	Abnormal
I	93/107 (87 %)	4/5 (80 %)
IIA	116/164 (71 %)	5/7 (71 %)
IIB	102/181 (56 %)	2/12 (17 %)
III	110/339 (32 %)	9/61 (15 %)
IV	5/39 (13 %)	3/58 (5 %)
Total	426/830* (51 %)	23/143 (16 %)

* Seventeen cases lost to follow-up excluded (2 %)

nation was performed, and also because most of these patients were in poor general condition and in terminal stages of their disease, which made them unacceptable for treatment and made further evaluation of little significance The urography was considered normal in 847 patients, and abnormality was observed in 143 cases, giving an incidence of 14 per cent

Eighty four per cent of the 990 patients with pretreatment intravenous urography received a full course of irradiation for carcinoma of the cervix uteri The patients who had a complete course were treated routinely by external irradiation followed by a single intracavitary application of radioactive sources in the majority of cases

External irradiation An exposure of 3 800 rad calculated at the mid plane of the pelvis was administered in eight weeks by means of anterior and posterior fields of 18 cm × 12 cm and sciatic fields of 8 cm × 10 cm with a 250 kVp unit, HVL of 2 5 mm Cu, and an SSD of 50 cm After 1959, a number of cases were treated with an Eldorado Cobalt 60 unit, 8 000 Ci, SSD of 100 cm, opposing fields, size 16 cm × 12 cm, exposures calculated at the mid pelvic plane, 4 300 rad delivered in six weeks

Intracavitary therapy Our technique for intracavitary application of radioactive sources has been fairly uniform It always follows the ex— [illegible]

[illegible] with three sources of 10 mg of radium (or the equivalent ^{60}Co) each, and a vaginal colpostat with two sources of 10 mg each If the apex of the vagina is narrow, then a long tandem with four or five sources of 10 mg each is placed with one or two sources protruding in the vagina

Table 4

Correlation of urography results, completeness of treatment and five year survival (in parenthesis)

Stage	Normal			Abnormal		
	Not treated	Incomplete	Complete	Not treated	Incomplete	Complete
I	–	3	106 (86 %)	–	–	5 (80 %)
IIA	1	7	161 (70 %)	–	1	6 (83 %)
IIB	1	11	172 (58 %)	1	1	10 (20 %)
III	8	47	291 (37 %)	5	12	44 (20 %)
IV	3	16	20 (25 %)	6	36	16 (19 %)
Total	13	84	750 (55 %)	12	50	81 (28 %)

Abnormal urographic examinations

The distribution of the cases by stage of the disease and incidence of normal and abnormal findings is presented in Table 1 The abnormalities were more frequent in advanced stages of the disease The incidence of abnormal findings was 5 % for combined stages I and II, as compared to 15 % in stage III and 60 % in stage IV

The abnormal urographies before treatment were classified as unilateral and bilateral, and graded as mild, moderate or severe hydronephrosis, and nonfunctioning kidney Table 2 shows the distribution of cases by stage of the disease and its correlation with unilateral or bilateral findings and degree of obstruction One hundred and six (74 %) of the alterations found were unilateral, of the 37 cases presenting bilateral abnormalities, 89 % were stage III or IV Eleven of 12 cases (92 %) with abnormal urography in stages I and IIA had either mild or moderate hydronephrosis Sixty per cent of those stages III and IV cases with abnormal findings at urography had either severe hydronephrosis or nonfunctioning kidney No function was observed in 68 cases, of which 93 % were stage III and IV

Results

The 5-year survival in patients with pretreatment urography is presented in Table 3 Out of 830 cases with normal findings, 426 survived 5 years (51 %), as compared to 23 out of 146 cases (16 %) with abnormal findings No significant differences were found in stages I and IIA, but marked differences in survival were seen in stages IIB, III, and IV In stage IIB, patients with normal urography had a 56 % 5-year survival, whereas that of patients with ureteral

Table 5

Five year survival in patients with abnormal urography

Stage	Unilateral	Bilateral	Total
I	3/4 (75 %)	1/1 (100 %)	4/5 (80 %)
IIA	5/7 (71 %)	—	5/7 (71 %)
IIB	2/9 (22 %)	0/3 (0 %)	2/12 (17 %)
III	9/47 (19 %)	0/14 (0 %)	9/61 (15 %)
IV	3/39 (8 %)	0/19 (0 %)	3/58 (5 %)
Total	22/106 (21 %)	1/37 (3 %)	23/143 (16 %)

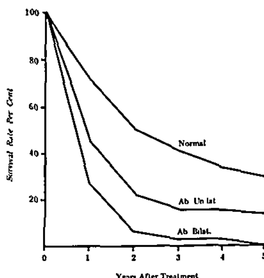
Table 6

Five year survival in patients with unilateral urographic findings

Findings	Stage					Total
	I	IIA	IIB	III	IV	
Hydronephrosis	2/2	4/5	2/4	2/8	0/3	10/22
Mild						
Moderate	1/2	1/1	0/1	4/20	1/10	7/34
Severe	—	—	0/1	1/3	0/1	1/5
No function	—	0/1	0/3	2/16	2/25	4/45
Total	3/4	5/7	2/9	9/47	3/39	22/106

obstruction was 17 %. In stage III, 32 % of the patients with normal findings survived 5 years, in contrast to 15 % of those with urinary tract obstruction. Thirteen per cent of stage IV cases with normal urography survived 5 years, as compared with 5 % of those with abnormalities.

Completeness of treatment was evaluated and it was found that 750 patients (88 %) with normal urography were able to complete their treatment, as compared with 81 patients (57 %) with abnormalities in the urinary tract. Eleven per cent of the patients with normal findings who received an incomplete treatment according to our standards survived 5 years. None of the patients with incomplete treatment of those with urinary tract involvement survived. In Table 4, completeness of treatment and 5 year survival in patients with normal and abnormal urography is shown. Fifty five per cent of the 750 patients with normal findings who received a complete course of irradiation survived 5 years. Only 28 % of the 81 patients with abnormalities survived 5 years, of these 74 % had mild or moderate unilateral hydronephrosis.



Survival of patients in stages III and IV with *normal urography, unilateral changes and bilateral changes*

In all stages significant differences were noted in the survival rate of patients with unilateral versus bilateral urinary tract obstruction In Table 5 the 5-year survival of patients with unilateral and bilateral obstruction is demonstrated All patients but one, in stage I, in the group with bilateral obstruction died, while 21 % of those with unilateral obstruction survived 5 years

Table 6 shows the correlation of the degree of urinary tract obstruction with stage of the disease and 5-year survival in patients with unilateral abnormalities Mild unilateral hydronephrosis seems to have little influences on prognosis, but a more advanced degree of ureteral obstruction is definitely related to poor prognosis

Bilateral urinary tract obstruction always has an adverse effect, regardless of the grade of obstruction Of 37 cases with bilateral obstruction only one patient, in stage I with mild hydronephrosis, survived 5 years

In view that urographic alterations are seldom found in stages I and II, and when present, have little influence on the prognosis of cases in early stages whereas the majority of cases with abnormal findings are in advanced stages of the disease, the survival of patients in stages III and IV with normal findings as compared with that of patients in the same stage of the disease but with unilateral or bilateral changes, is presented in the Figure At the end of 1 year, a marked difference in survival is noted, 71 % of patients with normal findings survived, while only 45 % of those with unilateral changes and 27 % of those with bilateral abnormal findings survived 1 year Thirty per cent of the patients with normal findings survived 5 years or more, whereas only 14 % of the patients with unilateral obstruction and none of those with bilateral obstruction were alive at 5 years

Table 7

Correlation between pathologic urography and prognosis

Reference	Stage	No of cases	Alterations	Five year survival
ALDRIGE & MASON (1950)	All	33	15 (34 5 %)	16 0 %
DEARING (1953)	All	327	97 (29 7 %)	*
SCHEWE & SALA (1959)	III IV	196	94 (48 0 %)	6 4 %
BURNS et coll (1960)	All	365	118 (32 3 %)	23 7 %
RHAMY & STANDER (1962)	All	305	43 (14 1 %)	**
BARBER et coll (1963)	All	503	105 (20 9 %)	8 0 %
KOTTMEIER (1964)	All	1402	268 (19 1 %)	32 8 %
PARKER & FRIEDMAN (1966)	All	217	45 (20 7 %)	0 0 %
ILIYA et coll. (1966)	III IV	222	50 (22 5 %)	2 0 %
MIDBOE et coll (1969)	All	541	92 (17 0 %)	***
WAGGONER & SPRATT (1969)	All	945	215 (22 8 %)	6 5 %
Present series	All	990	143 (14 4 %)	16 0 %

* 29 0 % one year survival
** 40 0 % one year survival
*** 16 3 % survival unspecified 1 to 5 years

Discussion

In carcinoma of the cervix uteri the bladder and the ureters are common sites of invasion by direct extension of the tumor If the bladder is involved, the tumor may reach the mucosa, and then the case is in stage IV, even though the neoplasm does not cause marked dysfunction The effect upon the ureters produces a partial or total occlusion resulting in hydronephrosis, or nonfunctioning kidney as the most advanced degree of ureteral obstruction, however, the involvement of this portion of the urinary tract was disregarded as not altering the stage of the disease until April 1970, when the General Assembly of the International Federation of Gynecology and Obstetrics (FIGO) established that the presence of a hydronephrosis or nonfunctioning kidney due to stenosis of the ureter by carcinoma permits the allotment of a case to stage III, even if according to the other findings the case should be alloted to an earlier stage This involvement is of great significance when we realize that in most patients who die with carcinoma of the cervix uteri, the cause of death is very often related to the urinary tract involvement inducing a renal failure (AUSTER & SALA 1940, BEHNEY 1933, DREXLER & HOWES 1934, GRAVES et coll 1936, MARCIAL ROJAS & MEIGS 1955, MORTON & DIGMAN 1952)

The incidence of ureteral obstruction before treatment in patients with carcinoma of the cervix uteri has been reported in several publications and ranges from 14 % to 48 % (Table 7) but the true incidence is unknown, because we lack data from large series of cases, and in reported pretreatment urographic results the examination was performed only on a limited number of cases treated The percentage of cases without pretreatment evaluation ranges from 3 % to 60 % in the published data (BURNS et coll 1960, KOTTMEIER 1964, PARKER & FRIEDMAN 1966) In our series, urography was not done in 96 cases (9 %)

It has been generally accepted that involvement of the urinary tract before treatment is an ominous prognostic finding, however the correlation of prognosis to stage, unilateral or bilateral involvement, and degree of ureteral obstruction has been sparsely evaluated (BARBER et coll 1963, ILIYA et coll 1966, MORTON & DIGMAN 1952, POMEROY 1947)

Correlating the results of urography with survival by stage, no significant differences were found in the 5-year survival between normal and abnormal for stages I and IIA, but in stages IIB and III the survival of patients with abnormalities is considerably diminished and statistically significant ($p < 0.01$) The 5-year survival of stage IIB patients with abnormal findings was 17 %, in sharp contrast to 56 % for those with normal findings Stage III cases with urinary tract involvement had a 15 % 5 year survival, while that of patients with normal findings was 32 %

In our series of 143 cases with abnormal findings, 106 cases (74 %) had unilateral and 37 cases (26 %) bilateral obstruction Unilateral mild hydronephrosis does not seem to influence prognosis, but moderate or severe hydronephrosis or renal failure has a definite prognostic significance Ten out of 22 patients (45 %) with mild unilateral hydronephrosis survived 5 years In contrast, only 7 out of 34 patients (20 %) with moderate hydronephrosis, 1 out of 5 (20 %) with severe hydronephrosis, and 4 out of 45 (9 %) with unilateral renal failure survived 5 years Except for one case, none of the patients with bilateral changes survived 5 years

The overall 5-year survival for the whole series is 51 % for patients with normal findings and 16 % for those patients with pathologic findings Our data are compared to the percentage 5-year survival reported in the literature in Table 7

The analysis of our cases of carcinoma of the cervix uteri indicates that urinary tract changes demonstrated at urography before treatment means a poor prognosis

Urography constitutes an objective method for evaluating the stage of the disease and should therefore be performed in all cases before treatment Urinary tract involvement is considered at present to be one of the factors which deter-

mine stage in carcinoma of the uterine cervix Urographic changes in early stages mean that these cases are to be classified as stage III Unilateral renal failure, or bilateral urinary tract involvement should perhaps be considered in future revisions an indication for classification as stage IV, along with the other criteria which are presently used

Acknowledgement

This work was supported in part by Public Health Service Grant No T1 CA 5169-08 G C de V is a World Health Organization and Pan American Health Association Fellow in radiation therapy and cancer

SUMMARY

An anlysis of urographies in 990 out of 1 086 patients with carcinoma of the cervix uteri has been made Pathologic changes were found in 143 cases, or an incidence of 14 % They were more often seen in stages III and IV The degree of ureteral obstruction was closely related to the stage of the disease Mild unilateral hydronephrosis had little influence on prognosis while more advanced degrees of obstruction resulted in poor prognosis No significant differences in survival were found in patients with normal or abnormal urography in stages I and IIA, but marked differences were observed in stages IIB, III and IV The overall 5 year survival for patients with ureteral obstruction was 16 %, as compared to 51 % for patients with normal findings

ZUSAMMENFASSUNG

Es wurde eine Analyse der Urographien bei 990 von 1086 Patienten mit einem Carcinom des Cervix uteri vorgenommen Pathologische Veränderungen fanden sich bei 143 Fallen oder eine Haufigkeit von 14 % Diese wurden hauliger in den Stadien III und IV gesehen Das Ausmass der Obstruktion der Ureteren war eng zum Stadium der Erkrankung gekoppelt Eine geringfugige einseitige Hydronephrose hatte geringfugigen Einfluss auf die Prognose wahrend eine starker ausgepragte Obstruktion zu einer schlechten Prognose fuhrte Keine signifikanten Unterschiede im Überleben von Patienten mit normaler und abnormaler Urographie in den Stadien I und IIA wurden gefunden, wahrend starke Unterschiede bei Patienten in den Stadien IIB, III und IV beobachtet wurden Die gesamte 5 Jahres Überlebensrate von Patienten mit Obstruktion der Ureteren betrug 16 % verglichen mit der von 51 % von Patienten mit normalen Befunden

RÉSUMÉ

Les auteurs ont etudie 990 urographies sur 1 086 malades atteintes de cancer du col de l utérus Ils ont trouve des modifications pathologiques dans 143 cas, ce quit fait une frequence de 14 % Elles étaient plus frequentes aux stades III et IV Le degre d obstruction urétérale etait etroitement en rapport avec le stade de la maladie L hydronephrose unilaterale discrète a eu peu d influence sur le pronostic, alors que les degres d obstruction plus avances ont donne lieu à un mauvais pronostic Les auteurs n ont pas trouve de difference significative dans la survie chez les malades qui avaient une urographie normale ou anormale aux stades I et IIA mais ils ont trouve des differences marquees aux stades IIB, III et IV Le taux global de survie a 5 ans pour les malades atteintes d obstruction ureterale a eté de 16 % a comparer au taux de 51 % pour les malades qui avaient une urographie normale

The incidence of ureteral obstruction before treatment in patients with carcinoma of the cervix uteri has been reported in several publications and ranges from 14 % to 48 % (Table 7) but the true incidence is unknown, because we lack data from large series of cases, and in reported pretreatment urographic results the examination was performed only on a limited number of cases treated The percentage of cases without pretreatment evaluation ranges from 3 % to 60 % in the published data (BURNS et coll 1960, KOTTMEIER 1964, PARKER & FRIEDMAN 1966) In our series, urography was not done in 96 cases (9 %)

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In our series of 143 cases with abnormal findings, 106 cases (74 %) had unilateral and 37 cases (26 %) bilateral obstruction Unilateral mild hydronephrosis does not seem to influence prognosis, but moderate or severe hydronephrosis or renal failure has a definite prognostic significance Ten out of 22 patients (45 %) with mild unilateral hydronephrosis survived 5 years In contrast, only 7 out of 34 patients (20 %) with moderate hydronephrosis 1 out of 5 (20 %) with severe hydronephrosis, and 4 out of 45 (9 %) with unilateral renal failure survived 5 years Except for one case, none of the patients with bilateral changes survived 5 years

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FROM THE DEPARTMENT OF RADIATION THERAPY, THE HOSPITAL FOR JOINT DISEASES AND MEDICAL CENTER, NEW YORK, N Y 10035, U S A

GROWTH RATE INVESTIGATION AND TUMOR LETHAL DOSE IN EWING'S SARCOMA

A W PEARLMAN

An estimate of a tumor s potential for malignant growth can be deduced from its duration, size and degree of histologic differentiation These parameters yield, at most an imprecise clinical index of tumor virulence COLLINS et coll (1956) published a more quantitative definition of the evolution and biologic behavior of malignant tumors This was the first of a series of observations on tumor growth rate and indicated its clinical importance Based on the serial observation of 46 individual pulmonary metastatic tumors in 15 patients with Ewing s sarcoma, a concept of tumor growth rate and doubling time was obtained Based on the response of 71 individual tumors in 20 patients, to different doses of irradiation, the spectrum of tumor lethal dosage for this tumor was defined

Experimental evidence that a tumor may exhibit a constant exponential growth rate during both its visible and invisible phase was presented by MOTTRAM 1935 1936) who induced cutaneous epitheliomas in mice by local application of tar Serial measurements of visible tumors were recorded graphically and the growth rate was found to be constant Backward extrapolation, through the invisible growth period, coincided with the first application of the irritant suggesting that these tumors could have arisen from a single cell and that the growth

Submitted for publ cation 6 December 1971

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Submitted for publication 6 December 1971

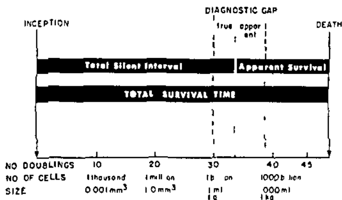


Fig. 1. Model of tumor growth of a hypothetical tumor illustrating the sequence of events when growth rate is exponential. The total survival time has been divided into preclinical (silent interval) and a clinical period from onset of symptoms to final status (apparent survival period). The preclinical period which may comprise as much as 75 per cent of the entire duration of the tumor, is the time required for a single cell 10 μ in diameter to attain a size of 1 cm diameter (approximately 30 tumor doublings). Increase in tumor size thereafter is quite rapid with each additional tumor volume doubling.

rate apparently constant in both the clinical and preclinical periods. This hypothesis of the development of a malignant tumor suggests that the tumor increases in volume in a geometric progression at a more or less constant rate throughout most if not all of its life. The rate of growth is expressed as the doubling time, the time required for a two-fold increase in volume (SPRATT & ACKERMAN 1961).

This simplistic concept of the evolution of malignant tumors has not gone unchallenged. SMITHERS (1968) has pointed out that there is no available experimental data on the growth rate of tumors of microscopic size and that the period of clinical observation from which many of the conclusions concerning tumor growth have been drawn, is after all only a small portion of the life of a tumor. Investigations of cell kinetics have demonstrated a discrepancy between the clinical doubling time and potential doubling time of tumors. The latter is calculated on the assumption that all mitosis produces two viable cells. These observed differences in growth rates may be explained by cell loss which occurs as a result of cell death, migration of cells outside the tumor or the inability of some daughter cells to reproduce (resting fraction) (BAGSHAWE 1968, FRINDEL et coll. 1968). GARLAND et coll. (1963) and GARLAND (1966) have stated that while exponential growth may not necessarily be present for the entire life of the tumor, it is reasonably well established that for moderate sized tumors (up to 2 cm in diameter) constant growth occurs over a significant part of a tumor's life. Even as a clinical approximation, the biomathematics of

exponential tumor growth helps clarify certain aspects of the behavior of human neoplastic disease and focuses attention on the long interval between the inception and the first visible manifestation of a tumor

A model of exponential tumor growth is illustrated in figure 1 (COLLINS et coll 1956 SCHWARZ 1961) The clinical diagnosis of carcinoma is only a single important event in the total course of the disease It has been preceded by an invisible preclinical phase and will be followed by a variable though shorter interval during which unrestrained growth or unsuccessful treatment will terminate in death The preclinical phase may occupy as much as three quarters of the entire life span of the tumor since it requires approximately 30 doublings in volume for a single cell, 10 μ in diameter, to reach a diameter of 1 cm, a size generally accepted as the smallest diagnosable tumor Should growth continue at the same rate it would require relatively few additional doublings for the tumor to become huge so that by the 45th doubling, tumor size is incompatible with the life of the host The clinical diagnosis is usually made some time after a tumor reaches diagnosable size There is usually a diagnostic gap between the earliest diagnosable tumor and the onset of symptoms This has been designated the true diagnostic gap The presence of tumor is not clinically suggested during this interval and it is not likely that this gap can be shortened in the interest of earlier diagnosis A second delay in diagnosis may occur between the onset of symptoms and the actual diagnosis and has been designated the apparent diagnostic gap This diagnostic delay can be shortened Taken together, the true and apparent diagnostic gaps usually occupy a relatively small portion of the life cycle of the tumor Considering the long preclinical period of growth, clinical diagnosis is not an early event

Material and Method Fifteen patients fulfilled the following criteria The diagnosis was histologically documented, all had measureable pulmonary metastases and sequential films were available for analyses The clinical course of each patient is illustrated in figure 2

Doubling times were calculated according to a formula proposed by GERSTENBERG (quoted by PHILIPPE & LE GAL 1968) which assumes that carcinoma cells divide at approximately equal and constant intervals

$$Dt \approx \frac{0\,3\,(t - t_0)}{\log dt - \log d_0} \qquad (1)$$

where d_0 and d_t are two successive diameters of a cancerous nodule observed at an interval of t days In this investigation only the first and last measured diameters were used to calculate the doubling time Minor variations in growth rate were disregarded Each patient had from one to six nodules Multiple

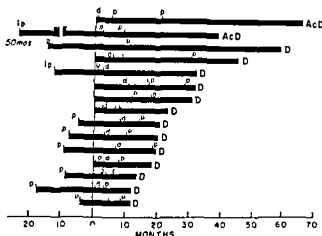


Fig 2 Clinical course of the 15 patients in this series Onset of symptoms (0) is the common starting point first clinical evidence of pulmonary metastasis (p) first diagnosis (d) and final status alive with disease (AcD) or dead (D) The growth rate of the first pulmonary metastasis was used to determine by backward extrapolation the probable time of inception of the first pulmonary metastasis (ip) In 9 patients inception of pulmonary metastasis preceded the onset of symptoms and in another patient preceded diagnosis One patient (second from top) had an unusually long preclinical period estimated to be 50 months from the inception of pulmonary metastasis to the onset of symptoms In the other patients the short pre clinical period reflected the rapid tumor growth

diameters were recorded for each nodule and the results averaged The same nodules were measured at different times and discrepancies adjusted by averaging the results

There are difficulties inherent to the accurate mensuration of pulmonary nodules Margins may blend into the surrounding lung parenchyma, ribs or mediastinum As adjacent nodules grow, they tend to become confluent Minor errors in measurement of large tumors are of little significance but assume some importance for small tumors

The average diameter of each nodule was plotted on semilogarithmic graph paper From the curves so constructed (Figs 6, 7, 8) it is possible to determine growth rate, the average growth slope and to observe the influence of various treatment modalities on tumor size

Clinical course The clinical course of the fifteen patients is illustrated in figure 2 with the onset of symptoms as the common starting point A number of significant events has been recorded chronologically diagnosis, the first radiographic evidence of pulmonary metastasis, and the status at last observation, dead or alive with disease The growth rate of the first pulmonary metastasis was calculated from measurements of tumor diameter on serial chest

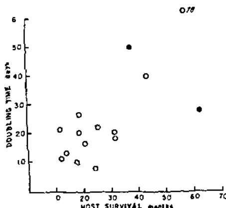


Fig 3 Survival in months correlated with the tumor doubling time of the most rapidly growing pulmonary metastasis Rapidly growing tumors (doubling time of 25 days or less) had a uniformly poor prognosis No patient survived beyond 32 months Three of the four longer survivors had doubling times of 40 days or longer (cf Fig 6) The number 78 refers to the doubling time in days of this patient's tumor This patient survived 55 months Open circles indicate patients dead solid circles patients alive at the time the investigation was completed

films, using formula 1 By backward extrapolation the probable time of inception of the first pulmonary metastasis was determined and recorded

Two patients are alive, both with disease The majority of patients are dead within 36 months of onset of symptoms The apparent diagnostic gap for the primary tumor (time of onset of symptoms to time of diagnosis) is relatively short 3 months or less in two-thirds of the patients, indicating a minimal delay in diagnosis and an aggressive clinical course Diagnostic delays of this order of magnitude probably have little influence on the subsequent course of the disease

The estimated time of inception of the first pulmonary metastasis preceded the symptoms in 9 patients and preceded the diagnosis in 10 There is a high probability that a patient with Ewing's sarcoma will develop pulmonary metastasis at some time in the course of the disease In this selected group of patients, the extrapolated data indicate that microscopic metastatic pulmonary disease occurred before clinical manifestations in some two thirds of the patients This aggressive biologic behavior precludes 'cure' for most patients, with available treatment methods

The relationship between tumor growth rate and survival is shown in figure 3 Rapidly growing tumors (doubling time 25 days or less) were associated with a rapid clinical course Eleven of 12 patients were dead within 36 months Three of the 4 patients who survived for longer intervals (including two still alive at

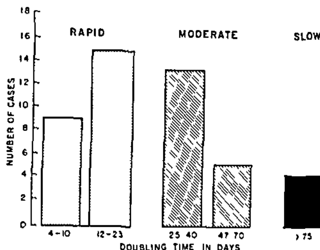


Fig. 4. Tumor doubling time of 46 individual tumors in 15 patients. Doubling times of 25 days or less are considered rapid, 26 to 75 days moderate and over 75 days slow. Over 50 per cent of tumors (24/46) were in rapid growth category. For convenience in analysis the rapid and moderate growth category have been further subdivided into two subgroups, respectively.

40 and 60 months) had slower growing tumors, as manifested by prolonged doubling times.

Doubling time of Ewing's sarcoma. The doubling time of 46 individual pulmonary tumors in 15 patients were calculated from serial measurements and recorded in figure 4. Tumors may be classified as rapidly growing (doubling time 25 days or less), moderate (25 to 75 days) and slow (greater than 75 days) (COLLINS et coll. 1956, BAGSHAWE 1968, PHILIPPE & LE GAL 1968). More than half the tumors fell into the rapid growth rate category and at least one-third had doubling times of 4 to 10 days, indicating extremely rapid growth. Only four tumors had doubling times in excess of 75 days (slow category). While the growth rate of different nodules in the same patient may vary, at least one rapidly growing tumor nodule was recorded for 11 patients. These observations of tumor growth rate are consistent with the known biologic aggressiveness of most Ewing's tumors.

Volume doubling times of 4 to 750 days have been recorded for various human tumors (COLLINS et coll. 1956, SCHWARZ 1961, SPRATT & ACKERMAN 1961, NATHAN et coll. 1962, GARLAND et coll. 1963, SPRATT et coll. 1963 a, b, SPRATT 1965, GARLAND 1966, BAGSHAWE 1968). GARLAND estimated that the average peripheral squamous cell carcinoma requires approximately 8 years to attain a diameter of 2 cm. PHILIPPE & LE GAL calculated the doubling times of mammary carcinoma, using patients with recurrent nodules in the operative scar, and

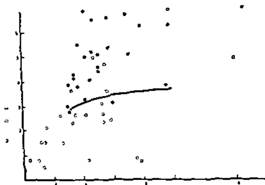


Fig 5 Scatter diagram of 71 tumor dose points culled from 20 patients The solid circles are successful doses and the open circles failures (tumor dose in krad) A free hand iso-effect curve has been constructed All doses below the curve were sublethal Some failures are, however observed at almost all dose levels The curve delineates a minimal tumor lethal dose range and is not necessarily optimal for irradiation of Ewing s sarcoma

found a bimodal distribution, one of quick growth, less than 25 days, and another of slow growth averaging 93 days Generally, rapid tumor growth is not usual for human tumors but occurs often in skeletal and soft tissue sarcomas, lymphomas and some embryonal tumors In one reported series, the mean doubling time of metastatic skeletal sarcomas, including Ewing's sarcoma was 32 days (SPRATT 1965) This compares with a mean doubling time of 30 days for this series

Tumor dose Seventy-one dose points culled from 20 patients have been assembled in a scatter diagram in figure 5 The solid circles represent doses that destroyed a primary or metastatic tumor With few exceptions, minimal observation period was one year or longer The successful dose points were obtained for the most part from th
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A free-hand iso-effect curve has been constructed through the smallest successful dose points and defines a dose range of approximately 3 000 rad in 10 days to 3 500 rad in 40 days This is the minimal dose range for arrest of tumor growth for more than one year in this group of patients All tumor doses below this zone failed but there were some failures at almost every dose level Based on the distribution of successes and failures, there is an approximately 75 per cent probability of destroying a tumor with doses larger than indicated by the curve

One clinical problem that arises in patients with Ewing s sarcoma is the

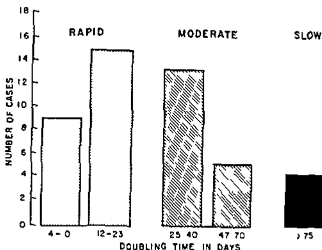


Fig 4 Tumor doubling time of 46 individual tumors in 15
or less are considered rapid 26 to 75 days moderate and o
of tumors (24/46) were in rapid growth category For cor
moderate growth category have been further subdivided into two subgroups respectively

40 and 60 months) had slower growing tumors, as manifested by prolonged doubling times

Doubling time of Ewing's sarcoma The doubling time of 46 individual pulmonary tumors in 15 patients were calculated from serial measurements and recorded in figure 4 Tumors may be classified as rapidly growing (doubling time 25 days or less), moderate (25 to 75 days) and slow (greater than 75 days) (COLLINS et coll 1956, BAGSHAWE 1968, PHILIPPE & LE GAL 1968) More than half the tumors fell into the rapid growth rate category and at least one-third had doubling times of 4 to 10 days, indicating extremely rapid growth Only four tumors had doubling times in excess of 75 days (slow category) While the growth rate of different nodules in the same patient may vary, at least one rapidly growing tumor nodule was recorded for 11 patients These observations of tumor growth rate are consistent with the known biologic aggressiveness of most Ewing s tumors

Volume doubling times of 4 to 750 days have been recorded for various human tumors (COLLINS *et coll* 1956, SCHWARZ 1961, SPRATT & ACKERMAN 1961, NATHAN et coll 1962, GARLAND et coll 1963, SPRATT et coll 1963 a, b, SPRATT 1965, GARLAND 1966, BAGSHAWE 1968) GARLAND estimated that the average *peripheral* squamous cell carcinoma *requires* approximately 8 years to attain a diameter of 2 cm PHILIPPE & LE GAL calculated the doubling times of mammary carcinoma, using patients with recurrent nodules in the operative scar, and

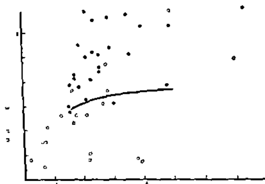


Fig 5 Scatter diagram of 71 tumor dose points culled from 20 patients The solid circles

... necessary optimal for irradiation of Ewing's sarcoma

found a bimodal distribution, one of quick growth, less than 25 days, and another of slow growth averaging 93 days Generally, rapid tumor growth is not usual for human tumors but occurs often in skeletal and soft tissue sarcomas, lymphomas and some embryonal tumors In one reported series, the mean doubling time of metastatic skeletal sarcomas, including Ewing s sarcoma was 32 days (SPRATT 1965) This compares with a mean doubling time of 30 days for this series

Tumor dose Seventy-one dose points culled from 20 patients have been assembled in a scatter diagram in figure 5 The solid circles represent doses that destroyed a primary or metastatic tumor With few exceptions, minimal observation period was one year or longer The successful dose points were obtained for the most part from ...

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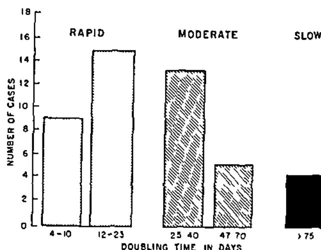


Fig 4 Tumor doubling time of 46 individual tumors in 15 patients Doubling times of 25 days or less are considered rapid, 26 to 75 days moderate and over 75 days slow Over 50 per cent of tumors (24/46) were in rapid growth category For convenience in analysis the rapid and moderate growth category have been further subdivided into two subgroups respectively

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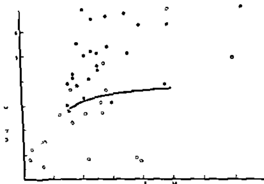


Fig 5 Scatter diagram of 71 tumor dose points culled from 20 patients The solid circles are successful doses and the open circles failures (tumor dose in krad) A free hand iso-effect curve has been constructed All doses below the curve were sublethal Some failures are however observed at almost all dose levels The curve delineates a minimal tumor lethal dose range and is not necessarily optimal for irradiation of Ewing s sarcoma

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Tumor dose Seventy-one dose points culled from 20 patients have been assembled in a scatter diagram in figure 5 The solid circles represent doses that destroyed a primary or metastatic tumor With few exceptions, minimal observation period was one year or longer The successful dose points were obtained, for the most part, from the irradiation of the primary bone tumor The open circles represent doses that were sublethal i e , persistent or recurrent tumor after irradiation

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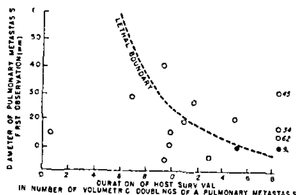


Fig. 6. The lethal boundary is the limit of survival of untreated patients measured in multiples of tumor volume doublings when the size of the pulmonary metastasis, at first observation, is known. There is a probality of only 0.05 that the untreated patient will survive beyond the lethal boundary based on the log normal distribution of growth rate of pulmonary metastasis. The life span of approximately one third of the patients in this group exceeded the lethal boundary. The patient who reached 92 volume doublings is now dead with disease. The other survivor, indicated by a solid circle, is still alive with disease and is now well beyond the lethal boundary. This would suggest that treatment benefited one in three patients. The figures adjacent to the circles on the right margin refer to the survival as measured in multiples of tumor doubling time. Open circles indicate patients dead; solid circles patient alive at the time the investigation was completed.

irradiation of pulmonary metastases. MARGOLIS & PHILLIPS (1969) irradiated the whole lung for pulmonary metastases and concluded that normal tissue tolerance ranged from 2 100 rad in 10 days to approximately 5 000 rad in 60 to 70 days. Two of their 7 patients with pulmonary metastases from Ewing's sarcoma achieved local control with relatively small doses (2 200 rad in 18 and 35 days, respectively.) The minimal lethal dose range of figure 5 for this series is greater than the whole lung tolerance, quoted above. No pulmonary nodules in our patients were destroyed with doses smaller than this lethal dose range. Irradiation of a segment of a lung with higher doses did achieve local control. *It would appear unlikely that one could sterilize pulmonary metastases by whole* lung irradiation and still remain within the tolerance of the normal tissues, in any but the most radiation sensitive Ewing's sarcomas.

Lethal boundary. The therapeutic efficiency of a treatment program may be evaluated by comparing the survival of treated patients with the maximum expected survival of untreated patients. The concept of a lethal boundary for untreated patients with pulmonary metastases was introduced by SPRATT et coll. (1963, 1964, 1965). It is a statistical correlation between measured rates of growth and duration of host survival. Maximum survival is recorded in multiples of tumor volume doublings when the size of the pulmonary metastases at the

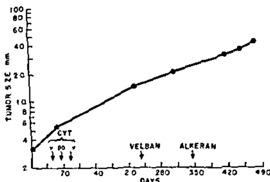


Fig 7 Male aged 5 Growth curve of a single pulmonary metastasis observed for 1 1/3 years
te of growth)
mm Cytoxan
growth rate
... from onset of symptoms and 18 months from first diagnosable pulmonary metastasis as measured in volume doublings not exceeding the lethal limit and no longer than might be expected in an untreated patient (probability 0 05)

first observation, is known The mathematic analysis of the lethal boundary concept is based on the observation that the growth rate of pulmonary metastases for a variety of tumors and the survival of patients follows a log normal distribution The probability is only 0 05, that a host with an untreated pulmonary metastasis would survive beyond the lethal boundary Conversely, there is a 95 per cent chance that the host will die before reaching the lethal boundary Since survival is measured in tumor volume doublings, the actual survival is the product of the number of doublings and the doubling time Reference to the original publication is recommended for the details of this unusual analytic method

The survival of the 15 patients in multiples of tumor volume doubling is plotted in figure 6 in relation to the lethal boundary The two patients who remain alive are designated by solid circles One-third of the patients survived beyond the lethal boundary For the remaining 10 patients, survival was no longer than might be expected for untreated patients Since therapeutic efficiency is measured in terms of tumor volume doublings rather than in actual survival time, the rate of growth of the tumor is not a factor in this analytic method, as it would be, if a stated time interval were chosen as the end point

The enhanced survival of one in three patients ...

... patients survived free of ... is no argument for therapeutic nihilism

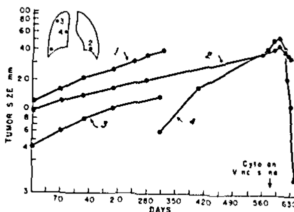


Fig 8

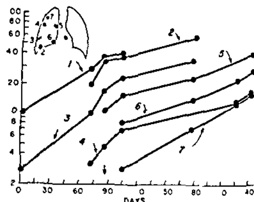


Fig 9

Fig 8 Female aged 8 Growth curves of four pulmonary nodules While there is considerable variation in growth rate of individual tumors (doubling times were 28 62 63 and 85 days respectively) (individual nodules tend to grow at a constant rate for long periods The response to chemotherapy with vincristine and cytoxan was a dramatic and rapid shrinkage of the pulmonary metastasis The patient is alive and functioning well 3 1/3 years after onset on maintenance chemotherapy despite the presence of pulmonary metastasis (In this figure and in Fig 9 each pulmonary nodule observed has been graphed individually The dots indicate a measurement of the diameter of the nodule on a serial chest film)

Fig 9 Growth curves of multiple pulmonary tumors The growth rate varied within a relatively small range (tumor doubling times 14 20 21 23 and 34 days respectively) Individual curves varied in growth rate but this was exponential for long periods

The tumor growth curves for three representative patients, plotted semilogarithmically, are illustrated in figures 7, 8 and 9 A straight line indicates a constant growth rate There are variations in the growth rate of different nodules in the same patient for similar observation periods There are also variations of growth rate of a single untreated nodule These differences in growth rate tend to be small and are most evident for small tumors, less than 1 cm in diameter, where even small errors in measurement are disproportionately exaggerated A more or less constant growth rate for long periods is usual

The effect of chemotherapy on tumor size is illustrated in figure 7 In this patient, treatment with vincristine and cytoxan was immediately followed by rapid shrinkage of the pulmonary tumors Another patient (Fig 8) had no objective tumor response to a variety of chemotherapeutic agents Both are alive with disease, for 3 and 5 years respectively No correlation could be established between the immediate tumor response and prognosis

Modest variations in growth rate notwithstanding the clinical implications of the theory of constant exponential growth seem valid Backward extrapolation to a time of inception of a tumor is a reasonable approximation in most instances,

and, if not mathematically exact, serves to emphasize the long preclinical phase during which the aggressive behavior of rapidly growing tumors may become manifest

Discussion

The biomathematic approach to the behavior of malignant tumors, as outlined above has been applied in this investigation to analyze the clinical course of a group of patients with metastatic Ewing s sarcoma Two observations may have applicability to malignant tumors generally A specific growth rate is characteristic of an individual tumor As suggested by COLLINS et coll , 'assigning a numerical value in the form of doubling time offers a more precise statement of rate of growth than descriptive phrases such as rapidly-growing or slowly-growing Secondly, the concept of origin from a single cell and subsequent exponential growth emphasizes the long preclinical period before diagnosis Early diagnosis is not an early event in the total life of a tumor This may explain the failure, at times, of early diagnosis to influence cure Treatment failures are apt to be due to undectected metastases, already present, though not clinically evident when treatment is begun, especially in malignant bone tumors

It is an accepted practice to report end results at stated times, usually 5 years or multiples thereof As a measure of therapeutic efficiency for certain growths, this may be questioned Long survivals in patients with slow growing tumors may only reflect a long doubling time It is suggested that comparisons of treatment results are more accurately reflected by recording survival in multiples of tumor doublings Unfortunately this information is not available for most primary tumors either because they are inaccessible to direct measurement or serial observations are curtailed by early treatment

The theory of exponential tumor growth has found clinical support in the concept of the period of risk This has led to an unique analysis of end results in certain childhood tumors Children with Wilms tumors who have survived symptom free following treatment for a period, equal to their age at onset plus 9 months of gestation, have rarely had a recurrence (COLLINS et coll 1956, KNOX & PILLERS 1958, POLLOCK et coll 1960) Similar observations have been recorded in neuroblastoma (KNOX & PILLERS 1958, SUTOW 1958, BODIAN 1959, POLLOCK et coll 1960), rhabdomyosarcoma (KNOX & PILLERS) and medulloblastoma (KIESEWETTER & MASON 1960) The 'period of risk' is based on the theory that viable tumor cells surviving after definitive therapy grow at more or less the same rate as the original tumor A recurrent tumor should become clinically manifest within an interval no greater than the longest possible time that the original tumor was present

The clinical course of our patients indicates that Ewing s sarcoma is a biologically aggressive tumor with a rapid growth rate and a propensity for early dissemination This is supported by the preponderance of tumors with doubling times of less than 25 days, by the high probability of microscopic pulmonary metastases before the primary tumor attains a diagnosable size, and by the short clinical course after onset

There is some evidence that an aggressive therapeutic approach which includes radiation and chemotherapy may prolong life Based on survival as measured in tumor volume doublings, one in three patients with pulmonary metastasis, had a survival significantly longer than the expected survival of untreated patients

Conclusions

The growth of tumors that lend themselves to serial measurements can be described in terms of doubling time, the time required for a twofold increase in volume *The doubling time characterizes the rate of growth which tends to be* more or less constant during the period of observation for most tumors of moderate size

The majority of pulmonary tumors in this series were rapidly growing (doubling time 25 days or less) Slow-growing tumors with a doubling time of 75 days or longer were unusual

Ewing s sarcoma is an aggressive tumor The great majority of patients are dead within 3 years of onset There is a high probability that microscopic pulmonary metastases will occur during the preclinical period, before onset or diagnosis

A suggested minimal tumor dosage, based on 71 tumor lethal dose points, ranges from 3 000 rad in 10 days to 3 500 rad in 40 days All doses below this range failed to destroy the tumor Some failures occurred with higher doses

Survival as measured in multiples of doubling time suggests that a significant prolongation of life in approximately one third of patients follows an adequate treatment program

Acknowledgement

I am most grateful to Dr Milton Friedman for reviewing and correcting the manuscript His critical comments were most helpful

Addendum

Since submitting this manuscript two additional patients with pulmonary metastases were treated Five pulmonary nodules in patient A had *tumor doubling time* of 4 4 5 7 7 and 14 days respectively Two pulmonary nodules in patient B had doubling times of 14 and 19

days respectively In both patients backward extrapolation of the growth rate of the first pulmonary metastasis suggested the presence of microscopic implants in the lung before onset of symptoms (Fig 2) The pulmonary nodules of patient A responded to irradiation, but not to chemotherapy with vincristine, cytoxan or actinomycin D Patient B s pulmonary metastases responded to both chemotherapy (one lung) and radiation plus chemotherapy (opposite lung) Patient A survived 1 year from onset He survived 25 tumor volume doublings from the first appearance of pulmonary metastases This exceeds his expected lethal boundary of 13 tumor doublings (see Fig 6) and suggests prolongation of life as a result of therapy Patient B is alive and well and to the present time has exceeded 25 tumor doubling times since onset of pulmonary metastasis

SUMMARY

Ewing s sarcoma is a biologically aggressive tumor with a rapid growth rate (tumor volume doubling time) and a propensity for early dissemination Investigation of growth rate of individual pulmonary nodules indicates an exponential (constant) growth rate for long periods Backward extrapolation of the growth curve of the first pulmonary metastasis suggests that the earliest pulmonary implant occurred before onset of symptoms or diagnosis in two thirds of the patients A minimal tumor lethal dose range is proferred below which dose levels no tumors were destroyed This range is between 3 000 rad in 10 days and 3 500 rad in 40 days

ZUSAMMENFASSUNG

Ewings Sarkom ist ein biologisch aggressiver Tumor mit einer raschen Zuwachsrate (Tumor Verdopplungszeit) und einer Neigung zu fruhzeitiger Streuung Untersuchungen der Zuwachsrate individueller Knotchen in der Lunge deuten auf eine exponentielle (konstante) Zuwachsrate wahrend langer Perioden Ruckwartige Extrapolation der Zuwachskurve der ersten Lungenmetastase deutet darauf hin dass bei 2/3 der Patienten das erste pulmonelle Implantat vor Einsetzten der Symptome oder vor der Diagnose aufgetreten war Man kommt zu einem letalen Tumordosisbereich, unter dem keine Tumoren zerstort werden Dieser Bereich l egt zwischen 3 000 rad in 10 Tagen und 3 500 rad in 40 Tagen

RÉSUMÉ

Le sarcome d Ewing est une tumeur biologiquement aggressive avec un taux de croissance rapide (temps de doublement du volume tumoral) et une propension à la dissemination precoce L etude du taux de croissance de nodules pulmonaires isoles indique un taux de croissance exponentielle (constant) au cours de longues periodes L'extrapolation retrograde de la courbe de croissance de la premiere metastase pulmonaire fait penser que la première greffe pulmonaire s est produite avant le debut des symptomes ou avant le diagnostic chez les deux tiers des malades L auteur propose une limite minimale de dose lethale pour la tumeur au dessous de laquelle aucune tumeur n a eté détruite Cette limite est entre 3000 rad en 10 jours et 3 500 rad en 40 jours

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FROM THE DEPARTMENTS OF RADIATION THERAPY AND RADIATION BIOLOGY, ST BARTHOLOMEW'S HOSPITAL AND MEDICAL COLLEGE, LONDON, ENGLAND

MICROFRACTIONATION

A particular type of pulsed irradiation in the treatment of mammary tumours in C_3H mice

J STONE and P J CHESHIRE

The rationale behind the development of most methods of fractionation in the treatment of tumours with ionising radiations has been based on two factors gross tissue response (normal and tumour) (DEL REGATO 1968, QUIMBY & MACCOMB 1937, STRANDQVIST 1944) and repopulation kinetics with gross cellular repair (FOWLER 1968, REGAUD 1922, CHESHIRE & LINDOP 1969) In general, these methods involved time intervals between fractions, of hours, days or weeks Other attempts have been based on physico-chemical responses at the molecular level with intervals of fractions of a second (KUNKEL & OBERHEUSER 1963) and ultimately limited by dose rate (MCWHIRTER 1936, TOWN 1968)

A time interval of the order of seconds based on metabolic function (CHANCE et coll 1965, HESS & CHANCE 1961) rather than hours, days or weeks seemed worthy of investigation A number of other factors suggest this. These include rate of oxygen consumption by normal tissues (WRIGHT & HOWARD-FLANDERS 1957) and the time for repair of DNA and other large molecules (KENNY & COMMONER 1969 SETLOW 1967) It was with some of these ideas in mind that the following attempt at a new method of fractionation which might be termed microfractionation was made and its therapeutic advantages investigated

Submitted for publication 23 May 1972

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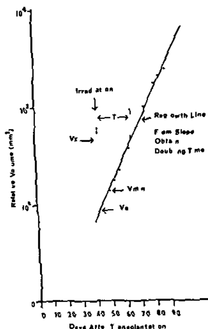


Fig 2 Growth curve for a typical irradiated tumour (relative volume on a log scale) Measurements before and after irradiation T = time for tumour to regrow to original volume at irradiation The doubling time is obtained from the slope of the curve in its exponential phase post irradiation V_I = volume at time of irradiation V min = minimum volume after irradiation V_S = surviving volume after irradiation and is the value of the volume at the intersection of the vertical from the time of irradiation and the regrowth line as shown

Each of the holes (S) took five seconds to travel across the collimator (Fig 1—SC, stationary cylinder) overlying the tumour which received approximately two rad per second, i e about ten rad in each five seconds The measured dose rate with a Baldwin Farmer dose meter and thimble chamber was approximately 65 rad per minute when the cylinder (RC) was rotating (pulsed irradiation), and 125 rad per minute when cylinder (RC) was stationary (continuous irradiation) The variation of dose across and through the tumour was less than 3 per cent These measurements were determined by means of both lithium fluoride and photographic emulsion dosimetry For a tumour dose of 3 000 rad the mouse received whole body irradiation of about 20 rad Accurate beam direction was achieved by putting the anaesthetised mouse on the perspex plate P (fixed with cellotape) inferiorly covered by perspex plate Q superiorly (giving the necessary 4 mm build up) with the tumour lying between a square etched on each plate (Fig 1) The superior plate Q fitted onto 4 brass posts fixed to P and this perspex holder (P, Q) thus formed fitted into a slot under SC so that it lay accurately under the hole S The set up was first checked without the mouse, aligning the cross wires of the ^{60}Co teletherapy unit and the etched crosses on the plates P and Q, all three of which had to coincide exactly

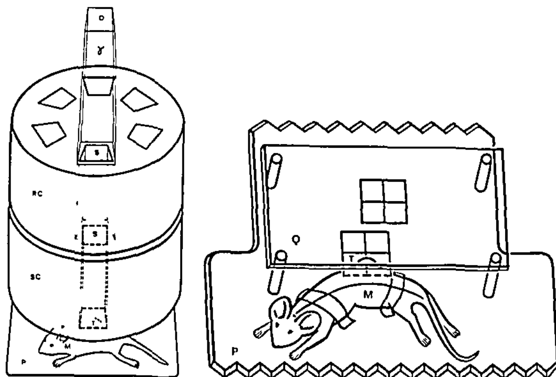


Fig 1 Diagrammatic representation of the apparatus set up to deliver pulsed or continuous irradiation to the tumour RC was driven by a clockwork motor D = diaphragm of ^{60}Co tele therapy unit, γ = γ rays, S = holes in RC and hole in SC RC = rotating cylinder SC = stationary cylinder, T = tumour, *M = mouse, P = inferior perspex plate Q = superior perspex plate*

Materials and Methods

Mice and transplanted mammary tissue Adult C_3H/HeJ male mice received first generation iso-transplants from a spontaneous mammary tumour arising in a female of the same strain Animals were housed eight to a cage Twice a week before and after irradiation, the three diameters of the tumour were measured using Vernier calipers and the product of the three diameters taken as the relative volume of the tumour This bears a linear proportionality relationship to actual tumour volume (CHESHIRE 1970) Tumours were irradiated when their relative volumes were in the range 250 to 800 mm^3

Irradiation In order to avoid the influence of inherent pulsed irradiations as found in linear accelerators (minipulses at *1/400 s for example) a cobalt 60 tele-*therapy unit was used to deliver the radiations to the tumour of an anaesthetised *mouse* To achieve pulses lasting five seconds each at intervals of five seconds a slowly rotating lead cylinder (Fig 1—RC) with appropriate holes was inserted between the cobalt 60 head and the tumour (T) in the flank of the mouse (M)

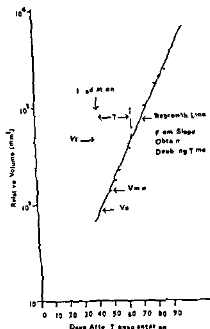


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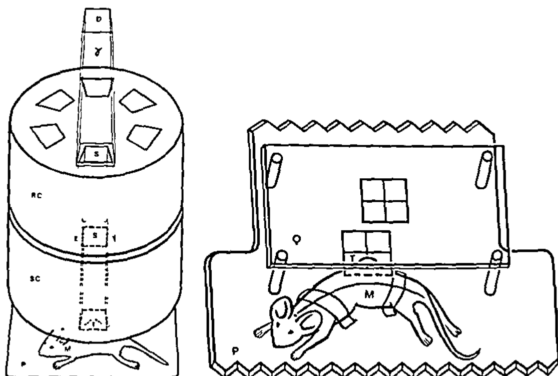


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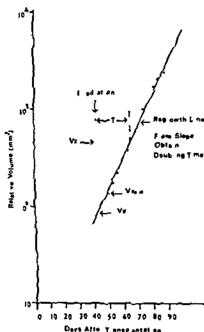


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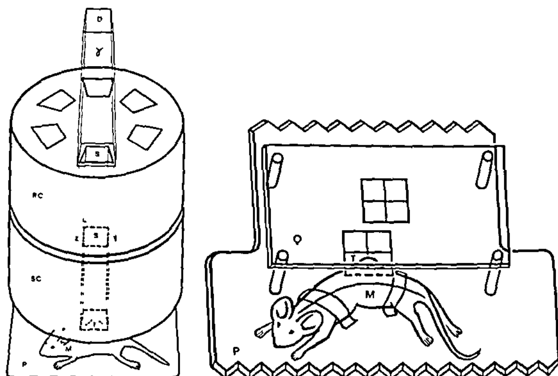


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5 2 mA/kg, a 10 mR film exposure equals 2 6 μC/kg This change to an SI unit may involve some difficulties for persons with insufficient experience in handling the results of physical measurement

The replacement of the *activity* unit 1 curie with the SI unit 1 per second, s^{-1}, seems to imply certain particular problems The SI unit is inconveniently small (Table) and the formation of the necessary multiple units will not be possible in the usual straight forward way For instance, the activity 10^6 s^{-1} (≈ 27 μCi) cannot be written Ms^{-1}, as according to the strict SI rules this means $(Ms)^{-1}$ or 1/Ms, which is equal to 10^{-6} s^{-1} The recommended symbol for 10^6 s^{-1} is then 1 μs^{-1}, one per microsecond, for 10^9 s^{-1} (≈ 27 mCi) it is 1 ns^{-1}, one per nanosecond, etc And the rate of change of a radioactive substance necessitates the use of notations such as 10 per microscond per second, 10 μs^{-1}/s Both examples may create confusion and increased risk of mistakes in radiation protection operations and in clinical practice

The safety of the patient in medicine and hospital care has to be carefully considered before the responsible national authorities prescribe a change in the present system of units A replacement of the special radiologic units with SI units implies several difficulties as shown by the examples above Some of these problems may well be solved by introducing new names for the derived SI units discussed in this context However, such new names are not desirable according to the principles of the CGPM, nevertheless this body recently accepted special names for the unit of pressure (1 pascal) and of electric conductance (1 siemens) Other measures may also be suggested in order to overcome the difficulties

The International Commission on Radiation Units and Measurements (ICRU) has as its principal objective the development of internationally acceptable recommendations regarding quantities, units and measurement of ionizing radiation and radioactivity The ICRU therefore invites and welcomes constructive comments and suggestions regarding the possible replacement of the special radiologic units 1 rad, 1 rontgen and 1 curie with derived SI units These comments may be transmitted to the Chairman, Dr H O Wyckoff, ICRU, 7910 Woodmont Avenue, Suite 1016, Washington, D C 20014, U S A the vice chairman, Prof A Allisy, Bureau International des Poids et Mesures, Pavillon de Breteuil, F 92 Sèvres, France, or the present writer, Prof Kurt Liden, Scientific Secretary of ICRU, Radiation Physics Department, University Hospital, S-221 85 Lund, Sweden

Table

Radiologic SI units

Quantity	Derived SI unit	Symbol	Present equivalent
Activity	1 per second	s^{-1}	$\approx 2.703 \times 10^{-11}$ Ci
Absorbed dose	1 joule per kilogram	$J \cdot kg^{-1}$	100 rad
Absorbed dose rate	1 watt per kilogram	$W \cdot kg^{-1} = J \cdot kg^{-1} \cdot s^{-1}$	100 rad $\cdot s^{-1}$
Exposure	1 coulomb per kilogram	$C \cdot kg^{-1}$	≈ 3876 R
Exposure	1 ampere per kilogram	$A \cdot kg^{-1} = C \cdot kg^{-1} \cdot s^{-1}$	≈ 3876 R $\cdot s^{-1}$

1 curie = 1 Ci = 3.7×10^{10} s^{-1} (exactly)
1 rontgen = 1 R = 2.58×10^{-4} $C \cdot kg^{-1}$ (exactly)
1 rad = (1 rd) = 0.01 $J \cdot kg^{-1}$ (exactly)

It follows from the strict SI rules that the numerical factors involved prevent *the adoption of these units as SI derived units*. However, in view of existing practice, the International Committee of Weights and Measures (CIPM 1969) *considered it preferable to keep the units curie, rontgen and rad for the time* being, to be used with the SI. Steps towards the eventual abandonment of the special radiologic units have, *however*, been taken by *the EEC countries*. The important benefits to be gained by world wide use of a common international and coherent system of quantities and units now calls upon a careful consideration of the 'be or not to be' of the special radiologic units.

Radiologic SI units and their equivalents expressed in the present special units are given in the Table. It is seen that the SI units imply substantial changes in the numerical values of the actual quantities. In the case of an *absorbed dose of 6400 rad to a patient a change to the corresponding* SI quantity value 64 J/kg should be relatively easy to perform. The difficulties *may be more marked if the absorbed dose rate has to be expressed in* watt per kilogram, W/kg, replacing rad/min, because the time unit is not explicitly given *in* the unit W/kg, which also masks the fact that the time unit is the second and not the minute. From a medical safety point of view it is also desirable that the absorbed dose and absorbed dose rate are expressed *in units containing* the same derived SI unit. The units J/kg and J/(kg · s) should then be the better choice, even *J/(kg · min) for absorbed dose rate could be considered*, as minute is retained for general use with the SI units.

The *quantity exposure* will be given a rather large SI unit, 1 C/kg, and an inconvenient conversion factor, 1 C/kg ≈ 3876 R. The main field for the use of the quantity exposure will be medical diagnostic radiology. For instance, the exposure rate 20 R/s corresponds to *0.0052 C/(kg · s) or 5.2 mC/(kg · s)* or

Table 1

Patient data obtained from hospitals in Finland, Norway, Iceland and Sweden

	Year of admission	No of patients
Finland		
University Central Hospital Helsinki (Prof M Sulamaa, Prof L Holsti, M Lindfors)	1952–69	74
Norway		
Rikshospitalet University of Oslo (O Knutrud, Prof E Poppe)	1947–68	37
Haukeland Sykehus, University of Bergen (P J Moe)	1961–69	16
Iceland		
Barnaspitali Hringsins, Landspitalinn, Reykjavik (Prof K Tryggvason)	1959–69	8
Sweden		
Sahlgrenska Sjukhuset University of Gothenburg (A Hultborn Prof B Rosengren)	1937–67	56
Akademiska Sjukhuset, University of Uppsala (Prof G Grotte, Prof B Nohrman)	1956–67	12
Lasarettet University of Lund (Prof M Lindgren, I Magnusson)	1940–68	37
Lasarettet University of Umeå (P Westling Prof L G Larsson)	1962–69	5
Other hospitals (O Hallberg Regionsjukhuset, Örebro, T Norin, Centrallasarettet Gavle)	1958–69	13
Radiumhemmet Karolinska Sjukhuset, Stockholm	1927–69	104

Material and methods

The patient data were obtained from hospitals in Finland, Norway, Iceland and Sweden (Table 1) The series comprised 362 patients, 188 males and 174 females, admitted between 1927 and 1969 with histologically verified nephroblastoma On admission, 128 of the patients were under 2 years of age and 64 under one year (Fig 1), in 3 patients the condition was noticed at birth, the oldest patient was 74 years of age The first sign was an abdominal mass in 71 per cent of the patients, haematuria in 2 per cent, and in the rest fever, gastric symptoms or urogenital infection All the patients were followed up For 335 of them the follow-up time was at least 3 years, reckoned from the end of the primary treatment and these were used for the analysis of the initial factors possibly influencing the cure rate The remaining 27 patients treated in 1968 and 1969 were controlled for less than 3 years All the patients classed as cured had no sign of recurrence from the time of the primary treatment until the end of the investigation in December 1970

FROM RADIUMHEMMET (DIRECTOR PROF J EINHORN), KAROLINSKA SJUKHUSET, 104 01 STOCKHOLM, AND THE STATISTICAL INSTITUTION, UNIVERSITY OF STOCKHOLM, 113 47 STOCKHOLM, SWEDEN

FACTORS INFLUENCING THE CURE RATE IN NEPHROBLASTOMA

A review of 335 cases

BERTA JEREB and GUNNAR EKLUND

An annual incidence in Sweden of two cases per million of the population (Cancer Incidence in Sweden, Annual Report 1967) makes nephroblastoma a rare disease Because of its varied biologic behaviour and the relatively large number of different forms of treatment the analysed series have often been too small to yield significant information (BOURNE 1967, BURCHER & EVEN 1968, BURGERT & GLIDEWELL 1967, CLATWORTHY 1969, FERNBACH & MARTYN 1966, HARVEY 1950, JEREB et coll 1969, JOHANSSON & ROSENGREN 1967, KLAPPROTH 1959, MAIER & HARSHAW 1967, SUKAROCHANA & KIESEWETTER 1966, VAETH & LEVITT 1963, WAGGET & KOOP 1970)

There has been steady improvement in the cure rate for *nephroblastoma* over the last years (ABESHOUSE 1967, D'ANGIO 1968, FARBER 1966, GROSS 1953, KLAPPROTH 1959, PARKKULAINEN et coll 1965, PEARSON et coll 1967, SCHWEISGUTH & SCHLINGER 1967, SCHWEISGUTH et coll 1970, SULLIVAN 1970, SUTOW et coll 1970, WILLIAMS 1964) The purpose of this investigation therefore was to examine the effect of various factors on the outcome of the disease and to ascertain which of them had an important bearing on the cure rate

Submitted for publication 11 October 1972

	— nodes Confined to the abdomen — except for metastases in the liver Tumours submitted to biopsy or bursting during the operation Inoperable tumours Tumours not completely removed
Stage IV	— Tumour spread to the liver or outside the abdomen
Bilateral tumours	— Nephroblastoma in both kidneys during lifetime
Not defined	— The information in 7 patients was too scant for useful retrospective staging to be possible In none of these, however, was there extra abdominal spread

The diagnosis was always confirmed by histology The specimens from Norway (53 cases) were reviewed previously (KNUTRUD 1961) and those from Finland (74 cases) in retrospect by RAPOLA. The histology features of 112 specimens from Sweden reviewed by SANDSTEDT are reported elsewhere (JEREB & SANDSTEDT 1973) In the remaining 123 cases it was necessary to rely on the original surgical and histology reports Only those renal tumours that were definitely classed as nephroblastoma or Wilms' tumour were included in the investigation The 19 patients with bilateral tumours and the 46 who primarily presented metastases were included only in the overall analysis of the series and reported elsewhere (JEREB 1971, 1973)

The patients were divided into two groups according to whether or not a cure was recorded — that is to say, whether the patient was alive and without any sign of the disease from the time of the initial treatment until the end of the investigation Patients dying and those alive at the end of the investigation but with metastases were not classed as cured, nor were 13 patients who developed metastases during the first year after the initial treatment and without sign of the disease more than three years after the last treatment of the metastases The 11 patients dying at operation are entered as 'not cured' in the tables presenting the overall results, and were excluded from the further analysis of the effects of the initial treatment

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for [illegible]

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far [illegible] out of the 41 in the first, 6 out of the 124 in the second and one out of the 170 patients in the third period In 6 of these 11 patients the tumour on admission was generalized, in 2 the tumour was assigned to stage III, in 2 both kidneys were affected and in one the stage could not be defined

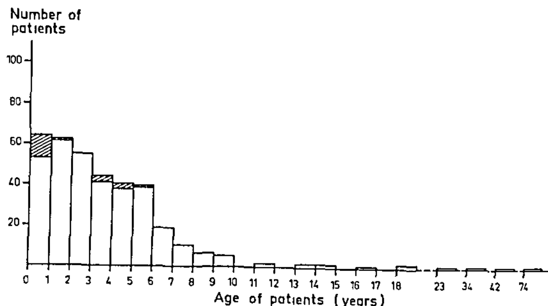


Fig 1 Age distribution of 362 patients with nephroblastoma admitted during 1927—69 ▨ bilateral tumours

Retrospective staging enabled the results of the different therapeutic methods to be compared The staging used, which is similar to that proposed by the National Wilms' Tumour Study Group (Stat Center, Seattle, Washington) is based on the pretherapy clinical examination, chest films, and surgical report as well as the histology. Clinical staging in the usual sense, based exclusively on the clinical examination before treatment, is impractical in cases of this type of tumour, the findings and other essential information were sometimes not available, although on the whole satisfactory It is thus not entirely a clinical staging, and will be referred to simply as 'staging' The main problem was the absence in some reports of information on the invasion of the blood vessels When, according to the surgeon's report, the tumour was well delineated and the capsule intact, and no information was available on the invasion of blood vessels, the tumour was assigned to stage I Whenever necessary and possible the interpretations of the available surgical and histology reports were discussed by personal contact

The stages are defined as follows

Stage I — Encapsulated tumour, completely removed with the renal capsule intact

Stage II — Local invasion beyond the renal capsule or involving blood vessels, including the vena cava but not adjacent organs or regional lymph nodes Tumour completely removed

Stage III — Tumour spread beyond the kidney, if only to regional lymph

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The series was divided into three periods according to whether the patient was admitted in 1927—49, 1950—59 or 1960—69 The second period roughly corresponds to the introduction of more active surgical and radiation treatment for the disease and the third period to the use of chemotherapy

Eleven patients had no treatment because the disease was considered to be too far advanced, they comprised 4 out of the 41 in the first, 6 out of the 124 in the second and one out of the 170 patients in the third period In 6 of these 11 patients the tumour on admission was generalized, in 2 the tumour was assigned to stage III, in 2 both kidneys were affected and in one the stage could not be defined

Forty-one patients were given only surgical treatment 13 (30 per cent) in the first, 20 (15 per cent) in the second and 8 (5 per cent) in the third period. In 5 of these 41 patients the stage could not be defined.

The transperitoneal approach was used in more than 90 per cent of the patients; only in the first period was an oblique flank incision employed.

Radiation therapy up to 1960 was always given with 200 kV roentgen units; after that time ^{60}Co units were used in 75 per cent of the patients. The radiation techniques with the different types of units were fairly uniform throughout the respective periods and for the various centres. The great majority of the patients were treated through two opposing portals from the xiphoid to the iliac crest. Since 1950 the treatment area has always been extended over the midline to encompass the whole vertebral body. In 10 per cent of the patients the area was limited to the tumour bed, but in 5 per cent the treatment was given through three portals, the calculated dose being at a maximum in the tumour and the para-aortic nodes, and falling off rapidly towards the periphery. About 20 per cent of the patients were treated through large portals covering the whole abdomen excluding the hip joints; in all the patients with a unilateral nephroblastoma the other kidney was shielded after 1 500 to 2 000 rad had been delivered.

The radiation dose was determined in retrospect in two ways. (1) When a treatment plan was available the dose was obtained from isodose curves. Direct measurements were sometimes made of the entrance and exit doses. The calculated dose — referred to here as the target tissue dose — was recorded in 55 per cent of all cases. The estimated error in its determination in this group was ± 10 per cent. (2) The dose delivered to each portal was recorded. The target tissue dose could then be estimated only on the basis of the type of therapy unit used, the number, size and position of the portals and the size (diameter) of the patient. The estimated error in this retrospective calculation was ± 20 per cent.

In both groups the mean dose in the target volume was calculated but no attempt was made to reconstruct the two- or three-dimensional dose distribution in the irradiated area. Most of the patients treated during the fifties and sixties belonged to group (1), whereas most of earlier subjects were in group (2).

The mean total maximum target dose for the preoperative and postoperative irradiation groups was about 2 600 rad and for those receiving only postoperative irradiation about 2 300 rad.

No patient in the first two periods was given actinomycin D. In the third period 78 out of 170 received this drug initially. 25 patients were given a single course beginning at the time of the operation and 53 patients had actinomycin D at 2 month intervals as a prophylactic measure for 15 to 24 months after operation. The total dose of actinomycin D was usually 60 to 75 µg/kg

body weight and was generally administered for 6 to 10 days per course Cyclophosphamide was sometimes applied intra abdominally at operation, no account has been taken of this treatment

The following data were recorded and tabulated for computer analysis (1) patient's age on admission, (2) stage of the disease at operation or initial treatment, (3) methods of treatment, (4) radiation dose administered, (5) chemotherapy, (6) stage of the tumour at the end of the follow-up period, (7) survival time after initial treatment, reckoned from the date of admission, (8) survival time after treatment for metastases, calculated from the time metastases were first diagnosed, (9) site of metastases, (10) interval between initial treatment and the appearance of the first metastases, (11) treatment of the metastases, (12) calendar year on admission

The material was first analysed manually and the results were tabulated An analysis of the various factors was first made on the whole material, which was then broken down into small groups for a more detailed analysis of the relative importance of each factor

Statistical methods

Choice of method The intention was to find (1) which of the factors, or combinations of factors, under investigation had a significant influence on the cure (and 2 year survival) rate, and (2) to what extent the variation in the cure (and 2 year survival) rate might be ascribed to the factors under investigation

Over the long period of time embraced by the investigation the factors under went modification and others possibly assumed significance For example, in the early years the patients were admitted at a more advanced stage of the disease, and radiation doses were smaller, moreover, later on, chemotherapy was added Thus, an analysis of each factor separately might, for instance, present strong correlation between chemotherapy and an improvement in the cure rate, irrespective of whether or not this improvement was due to other factors, such as higher radiation doses or admission at an earlier stage of the disease

The material in a retrospective investigation like the present one is not ideally amenable to statistical analysis by any method In this kind of series there is bound to be appreciable interaction between factors having a bearing on the cure and survival rates Furthermore, factors that exert a simultaneous influence on the outcome often cannot be isolated It was thus considered that the most suitable approach to the analysis of this series was to apply a modification of the Automatic Interaction Detection method (AID) (SONQUIST & MORGAN 1970)

In AID analysis a series of independent variables can be simultaneously examined in relation to one dependent variable The main advantage of the method is its ability to detect interaction effects Another lies in the way in which

Table 2

The predictors, number of possible splits and test values used in the analysis

Predictor	Variable type*	Number of possible splits for each predictor—N_i**	Limiting value $\chi^2_{\alpha_i}$	$\alpha_i = 0.01/N_i$
Calendar year on admission	M	26	3.55	0.000385
Patient's age on admission	M	17	3.44	0.000583
Stage of the disease at primary treatment	M***	4	3.02	0.002500
Method or primary treatment	F	491	4.27	0.000020
Radiation dose at primary treatment	M	4	3.02	0.002500

*M = Monotonic, F = Free

** Only splits that would result in two groups, each consisting of more than 10 observations are considered

*** Seven patients were not recorded for this variable. For this predictor to be monotonic it was necessary to re-code it as two new monotonic predictors (EKLUND & GAVATIN 1972)

the results are presented. The AID analysis is performed by a stagewise splitting of the original series into pairs of subgroups forming a 'tree'. The progress of the analysis can be followed step by step, since it is presented, together with the results, in a readily surveyable graphic form.

Variables. An examination was made of the influence of the recorded factors (predictors) first on the cure rate (result variable), and then on the 2-year survival rate. The result variable was dichotomous (cured, non-cured) and the results are presented as the percentages of cured patients and the 2-year survivors, respectively (Figs 7, 8).

The factors used as predictors are presented in Table 2. The predictors in the AID analysis may be monotonic or free. A monotonic predictor consists of a continuous series of the properties of the same factor — for example the patient's age. The order of the coded classes is maintained throughout the division process. Two subgroups can be formed only by dividing the series at one particular age. A monotonic predictor with K classes can divide a parent group in (K-1) ways, thus one with the four classes 1, 2, 3, 4 can divide the parent group in the three ways:

(1) against (2, 3 and 4)
(1 and 2) against (3 and 4)
(1, 2 and 3) against (4)

A free predictor consists of classes composed of different properties of the same factor — for instance, methods of treatment The classes might be rearranged during the division process The subgroups are then formed by selecting the most suitable classes irrespective of the their original position in the series A free predictor having K coded classes may therefore divide a parent group in $(2^{K-1}-1)$ ways For example, a free predictor with the four classes 1, 2, 3 and 4 can split a parent group in 7 different ways as follows

(1) against (2, 3 and 4)
(2) against (1, 3 and 4)
(3) against (1, 2 and 4)
(4) against (1, 2 and 3)
(1 and 2) against (3 and 4)
(1 and 3) against (2 and 4)
(1 and 4) against (2 and 3)

The number of potential ways of dividing a group for a free predictor increases rapidly with the number of classes in the series

Procedure The AID analysis is performed in the following way

1 (a) For each of the 5 predictors an attempt is made to split the original (parent) group into two subgroups Many such splits are possible, and the one chosen as a candidate is that which produces a pair of subgroups differing maximally from each other (see (b) below) as regards the result variable

(b) The resulting 5 pairs of subgroups are tested for their statistical significance The pair chosen is the one for which the difference presents the highest level of significance

(c) The parent group is split into the chosen pair of subgroups, the corresponding predictor being defined as the one with the greatest influence on the variability of the result variable

2 Each of the two subgroups chosen is then treated as a parent group and is split in the same way

3 The procedure is repeated and at each stage the predictor having the greatest influence on the variability of the result variable is chosen for the split

4 The procedure is terminated when none of the predictors attains the level of significance

5 The results of the analysis are presented as a 'tree' composed of pairs of subgroups, the differences between which are statistically significant

Splits that would result in subgroups containing less than 10 patients were omitted in this investigation

The level of significance The χ^2 test was used for examining the difference between the percentages into two subgroups The deviation of the value of χ^2 in

Table 2

The predictors, number of possible splits and test values used in the analysis

Predictor	Variable type*	Number of possible splits for each predictor — N_i**	Limiting value $\chi^2_{\alpha_i}$	Limiting value $\alpha_i = 0.01/N_i$
Calendar year on admission	M	26	3.55^2	0.000385
Patient's age on admission	M	17	3.44^2	0.000588
Stage of the disease at primary treatment	M***	4	3.02^2	0.002500
Method of primary treatment	F	491	4.27^2	0.000020
Radiation dose at primary treatment	M	4	3.02^2	0.002500

*M = Monotonic, F = Free

** Only splits that would result in two groups, each consisting of more than 10 observations are considered.

*** Seven patients were not recorded for this variable. For this predictor to be monotonic it was necessary to re-code it as two new monotonic predictors (EKLUND & GAVATIN 1972).

the results are presented. The AID analysis is performed by a stagewise splitting of the original series into pairs of subgroups forming a 'tree'. The progress of the analysis can be followed step by step, since it is presented, together with the results, in a readily surveyable graphic form.

Variables. An examination was made of the influence of the recorded factors (predictors) first on the cure rate (result variable), and then on the 2-year survival rate. The result variable was dichotomous (cured, non-cured) and the results are presented as the percentages of cured patients and the 2-year survivors, respectively (Figs 7, 8).

The factors used as predictors are presented in Table 2. The predictors in the AID analysis may be monotonic or free. A monotonic predictor consists of a continuous series of the properties of the same factor — for example the patient's age. The order of the coded classes is maintained throughout the division process. Two subgroups can be formed only by dividing the series at one particular age. A monotonic predictor with K classes can divide a parent group in (K-1) ways, thus one with the four classes 1, 2, 3, 4 can divide the parent group in the three ways:

(1) against (2, 3 and 4)
(1 and 2) against (3 and 4)
(1, 2 and 3) against (4)

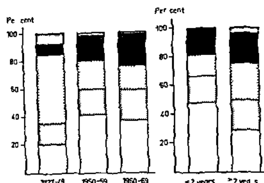


Fig 3

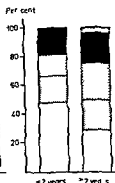


Fig 4

Fig 3 Stage distribution for three periods □ stage I ⊏ stage II □ stage III ▮ stage IV ■ bilateral tumours ⊏ stage not defined

Fig 4 Stage distribution by age (For symbols see Fig 3)

level of significance the probability 0 05 was shared between the 5 predictors, each being allotted a probability of 0 01 The 5 predictors were thus tested at the same 1 per cent level of significance and the criteria were sharpened

A value of a was also calculated for each predictor, the value a_i for the predictor i being defined as $0\ 01/N_i$, where N_i is the number of possible splits for the predictor i The criteria of significance for each predictor were thus adjusted to the probability of random splits, which varies according to the predictor The values of a for the 5 predictors appear in Table 2

The testing procedure N values of γ^2 for the predictor i appear in the observed series If the predictor is to give rise to a split the largest of these values must exceed $\gamma^2_{a_i}$ If no value of γ^2 for a predictor exceeds its limiting value (χ^2_a) no split can be performed at the specified level of significance If only one predictor reaches the level of significance it will produce a split into two subgroups If two or more predictors exceed their limiting values a lower value for the probability than 0 05 is chosen and new values of a are computed If more than one predictor still exceeds its limiting value the procedure is repeated for still lower values of probability until only one predictor reaches the extra sharpened level of significance and the split is performed

Coefficient of determination The coefficient of determination indicated in the figures of the AID tree denotes the percentage of the variations of the dependent variable that is accounted for by the predictors It was calculated from the expression

$$R^2 = 1 - \frac{\sum Y_1(N_1 - Y_1)/N_1}{Y_0(N_0 - Y_0)/N_0}$$

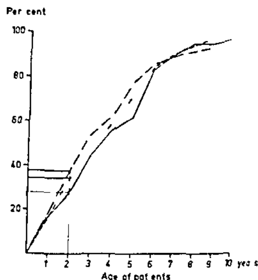


Fig. 2. Cumulative age distribution of patients with nephroblastoma in the three periods ——— 1927—49 — - — 1950—59 - - - 1960—69

the tested groups from the chosen limiting value of χ_α was thus examined. The differences in those pairs of subgroups defined by a value of χ greater than the value of χ^*_α were regarded as statistically significant, that is to say the probability that the difference in the percentages of the two subgroups was purely random was less than at the chosen level. In the conventional χ test the value of α defines the level of significance, it may, however, be specified in different ways as shown below. The formula used was Prob ($\chi \geqslant \chi^*_\alpha$) $= \alpha$. A critical value of χ corresponding to a specified value of α (converted to a percentage) occurs in the tables of the percentiles of the χ distribution.

The probability of a random split and calculation of α. The value of α is 0.05 in the conventional way of testing the subgroups for statistical significance at the 5 per cent level. In the present AID analysis there are 542 possible ways (see Table 2) of splitting the original group into pairs of subgroups. With such a number a chance exists that a split will occur at random — that is to say the predictor will have no influence on the result variable. Further, the probability of random splits for a predictor increases with the number of possible splits, for instance, for the predictor methods of treatment the possible number of splits is 491, against only 4 for the predictor stage of the disease. The conventional way of testing statistical significance of the subgroups takes no account of the possibility of random splits and the difference between the various predictors as regards the numbers of possible splits they may produce (EKLUND & GAVATIN 1972).

The purpose of modifying the AID analysis was to sharpen the criteria of significance and to reduce the probability of random splits. To ensure a 5 per cent

Table 3

Cure rate for the three admission periods 335 patients

Period	No of patients	Cured	
		No	%
1927–49	41	3	7
1950–59	124	36	29
1960–67	170	60	35
Total	335	99	30

Table 4

Cure rate for the groups under, and at least, 2 years of age on admission 335 patients

Age	No of patients	Cured	
		No	%
< 2 years	122	59	48
≥ 2	213	40	19

Table 5

Cure rate against stage at primary treatment 335 patients

Stage at primary treatment	No of patients	Cured	
		No	%
I	121	66	55
II	59	21	36
III	83	8	10
IV	46	0	0
Bilateral tumors	19	4	(21)
Not defined	7	0	
Total	335	99	

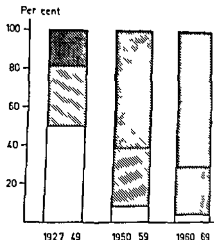


Fig 5 Radiation doses at initial treatment delivered during the three periods □ < 1 000 rad ▨ 1 000 to 2 000 rad ▩ ⩾ 2 000 rad

Where N_j is the total number of patients, Y_j the number of cured (surviving) patients in the terminal group and N_0 and Y_0 are the corresponding values in the original group

Results

Some factors changed with the passage of time The patients admitted in the later periods tended to be younger, 29 per cent of those from the first period were under 2 years old although this rose to 37 per cent for the last period (Fig 2) The patients in the later periods also tended to be in an earlier stage (Fig 3) and the radiation dose was higher (Fig 5) The disease was often more advanced in the older patients (Fig 4)

Of 335 patients, 99 were cured, 31 were alive at the end of the follow-up period but had metastases, and 205 had died

Eleven patients died during the operation or shortly after Cardiac arrest was the cause of death in 7 patients, 3 of whom were premature babies operated on during the first few days of life and 2 were assigned to stage IV Sepsis and bronchopneumonia were the respective causes of death in one patient each Two patients who had locally advanced tumours died of intra-abdominal bleeding

Of the remaining 194 patients all but one died from nephroblastoma The exception was a boy who was 6 months old on admission, had no recurrence after the initial treatment and died when he was 21 years old from a huge intrathoracal chondrosarcoma in a rib within an area irradiated 20 years previously

There was an improvement in the cure rate over the three periods (Table 3) As in most other investigations (EMMANUEL 1969, FLEMING & JOHNSON 1970, LATTIMER & CONWAY 1968), the cure rate was lower for the patients over 2 years of age and closely correlated to the stage of the disease (Tables 4, 5)

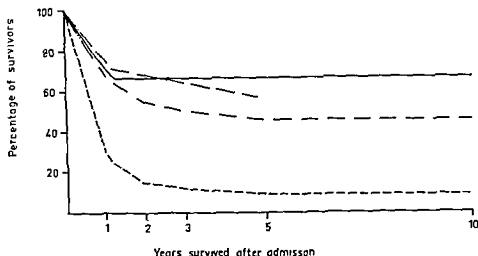


Fig 6 Survival according to methods of treatment, stage I to III and stage not defined — — — surgery only (35 patients), — — surgery and irradiation (125 patients), — — surgery irradiation and actinomycin D (57 patients), ——— irradiation, surgery and irradiation (20 patients)

received actinomycin D, but this was given to one patient of the 'surgery only' and 'surgery and preoperative irradiation' groups The patients not treated at all had stage III tumours None of the 10 patients receiving only irradiation was cured, one of these 10 belonged to stage II and 9 to stage III Surgery alone also produced poor results, only 4 out of 19 patients with a stage I tumour at operation were cured, and there were no survivors among the 9 in stage III, the 3 in stage II or the 4 patients in whom the stage was not defined In 3 of the 7 pa-

Table 8

Effect on cure rate of preoperative irradiation and actinomycin D 206 patients of stages I to III and stage not defined

Primary treatment	Without actinomycin D			With actinomycin D		
	No of patients	Cured		No of patients	Cured	
		No	%		No	%
Surgery + irradiation	125	47	38	57	28	49
Irradiation + surgery + irradiation	20	12	(60)	4	2	

Table 6

Cure rate for the groups with and without actinomycin D. 335 patients

Primary treatment	No of patients	Cured	
		No	%
Without actinomycin D	257	67	26
With actinomycin D	78	32	41

Table 7

Cure rate against type of primary treatment; 261 patients of stages I to III and stage not defined

Primary treatment	No of patients	Cured	
		No	%
No treatment	3	0	
Irradiation only	10	0	
Surgery only	35	4	11
Irradiation + surgery	7	2	
Surgery + irradiation	182	75	41
Irradiation + surgery + irradiation	24	14	58
Total	261	95	36

Eight patients with a stage II tumour had malignant invasion of the vena cava at operation; 4 of these were cured. In 21 of the patients with stage III metastases in the regional lymph nodes were evident but were probably not all removed at operation, the primary tumour, however, was completely extirpated Only one of these patients was recorded as cured

The cure rate in patients given actinomycin D was better than in those not so treated (Table 6).

The exclusion of the patients with bilateral tumours, stage IV patients and those dying at operation left 261 patients with the tumour in stage I, II or III or where the stage was not defined on admission.

The survival and the cure rate for the various methods of treatment are presented (Fig. 6, Table 7) None of the patients treated only by irradiation

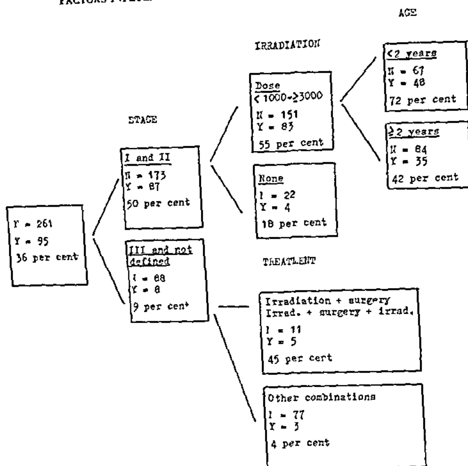


Fig 7 Cure rate in 261 nephroblastoma patients N = number of patients Y = number of patients cured $100 \frac{Y}{N}$ per cent Coefficient of determination 28 per cent

tion existed between several of the factors — for instance, between tumour stage and age, between radiation dose and supplementary chemotherapy — a more correct assesment would presumably have been obtained from an analysis in which several factors were considered simultaneously, tree analysis was accordingly performed

The 'tree' appears in Fig 7 The analysis covered those 261 patients belonging to stages I, II and III and stage not defined, who survived the operation Thirty-six per cent of these were cured, that is to say there was no sign of recurrence for at least 3 years after the initial treatment, thus 64 per cent were not cured

Table 9

Cure rate against radiation dose (rad) 261 patients

Radiation dose	No of patients	Cured	
		No	%
No irradiation	38	4	10
< 1000	19	5	(26)
1000–2000	55	24	44
2000–3000	79	31	39
> 3000	64	31	48
Dose unknown	6	0	

tients in Table 7 given only irradiation before the operation the tumour was encapsulated and completely removed In the other 4 patients the disease was in stage III One patient from each of these two groups survived Cures were recorded in 58 per cent of the patients receiving both pre- and postoperative irradiation, and in 41 per cent of those having only postoperative radiation therapy The cure rate was apparently better in those of the former group than in those given radiation therapy only after the operation and actinomycin D (Table 8). Where the radiation dose to the tumour was below 1 000 rad the patients did poorly (Table 9).

The outcome for the patients under one year of age was relatively good in all stages, 69 per cent being recorded as cured (Table 10)

The influence of not more than one or two factors on the cure rate was investigated simultaneously in the results described Since an appreciable correla-

Table 10

Cure rate against stage and type of primary treatment 42 patients under 1 year old Six patients died at operation

Primary treatment	Stage I		Stage II		Stage III		Total	
	No of patients	Cured	No of patients	Cured	No of patients	Cured	No of patients	Cured
Surgery only	3	1	1	0	2	0	7	1
Surgery+irradiation	15	13	5	3	4	3	24	19
Surgery+irradiation +actinomycin D	8	7	2	2	1	0	11	9
Total	26	21	8	5	7	3	42*	29

* In one patient the stage was not defined

Table 11

Condition of 8 surviving stage III patients at primary treatment pre and postoperative radiation dose (rad) and drug therapy Nephrectomy performed in all patients

Sex	Age (mos)	Stage at primary treatment	Radiation dose Preop	Postop	Drug therapy
M	24	Burst at operation Invasion of blood vessels pancreas colon	1 650	1 100	Cyclophosphamide intraabdominal ly
F	20	Burst at operation Invasion of blood vessels	400	2 400	*
M	6	Burst at operation Encapsulated	800	1 000	*
M	12	Attached to adjacent organs at first explora tory laparotomy	1 500	1 400	*
M	48	Burst at operation Invasion of blood vessels	—	2 500	*
M	6	Inoperable, fixed large tumour at first exploratory laparotomy	900	—	*
M	8	Burst at operation Encapsulated	—	2 400	
M	60	Burst at operation Invasion of lymph nodes	—	1 000 – 2 000	Actinomycin D multiple courses

* Macroscopic complete removal of tumour

The combined group of patients of stage III and stage not defined was also divided for 11 of those for whom surgery or surgery and postoperative radiation therapy were supplemented by preoperative irradiation the cure rate was 45 per cent against 4 per cent for the 77 patients with all the other combinations of treatment The 8 survivors who all had stage III tumours had in common none of the other factors under examination (Table 11)

Two-year survival — the next tree in Fig 8 — was created by replacing the cure rate with 2 year survival as the dependent variable Fifty one per cent of the 261 patients survived this period The differentiation into subgroups is similar to that for the cure rate tree Here, too, the first division was into stage I + stage II and stage III + stage not defined, the 2 year survivals were 66 and 19 per cent respectively The next division of the stage I + stage II group was into a group in which the patients had not received irradiation and had a poorer 2-year survival 32 per cent) than the rest (72 per cent) The latter group was further split into stage I and stage II, with respective survival rates of 81 and 55 per cent The group stage III and stage not defined was divided according to whether surgery and subsequent radiation therapy were supplemented with preoperative irradiation or chemotherapy (2 year survival 45 per cent) or not (12 per cent)

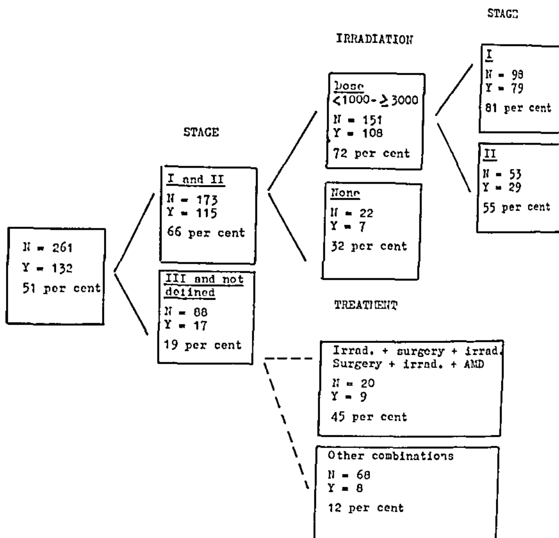


Fig 8 Two-year survival rate in 261 patients AMD = Actinomycin D N = number of patients, Y = number of survivors 100 $\frac{Y}{N}$ per cent Coefficient of determination 30 per cent The split indicated by the broken lines is not significant

The first division, i e. the one giving the greatest contrast — was made between the two pairs of factors: stage I + stage II and stage III + stage not defined The respective cure rates were 50 and 9 per cent. Stage I + stage II was divided into patients who were treated with irradiation and those who were not, the respective cure rates were 55 and 18 per cent

The group with a more favourable outcome was then divided according to whether the patients were less than or at least 2 years old on admission; the respective cure rates were 72 and 42 per cent.

treated in the third period when they also generally received higher doses of irradiation, and when on admission the tumours were probably at a lower stage

Conclusions

The stage of the tumour was the most important single factor governing the outcome The cure rate was significantly lower for patients with stage III tumours than for those with stages I or II The difference in the cure rate for the patients with stage I and stage II tumours was not statistically significant

Age was of importance in stages I and II, in those patients who were initially treated with irradiation the cure rate was better for those under two years of age than for the more elderly

The relative effect of surgical methods on the cure rate could not be determined

Irradiation treatment (1) The cure rate was significantly higher for those stage III patients in whom surgery alone or surgery and subsequent radiation therapy were supplemented by preoperative irradiation than for those not so treated (2) The cure rate was significantly higher for stage I and stage II patients who received radiation therapy than for those who did not (Fig 7)

Actinomycin D The survival rate was higher (but not statistically significant) for stage III patients when the surgery and subsequent radiation therapy were supplemented by preoperative irradiation or actinomycin D (Fig 8) Th[illegible]

[illegible]gation adduced no further evidence of an effect of actinomycin D on the cure rate

The year of admission failed to emerge as bearing influence As the stage of the disease at the first treatment is the most important prognostic factor, the improvement in the results during the fifties and sixties may be attributed in part to an increase in the proportion of early-stage tumours (Figs 7, 8)

Acknowledgements

This investigation was supported by the Swedish Cancer Society and the Cancer Society of Stockholm

SUMMARY

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[illegible]
[illegible] date the factors influencing the prognosis.

Discussion

A retrospective investigation of this kind with the patient data assembled from different centres and various periods and based on the surgeons' and pathologists reports makes accurate staging difficult. The system of staging of the nephroblastomas in the present investigation was devised to fit these particular conditions. Drawback of a staging system based to some extent on the surgical report is that the tumour may diminish in size as a consequence of the preoperative irradiation. It is probable that some of the 31 patients receiving such irradiation would have been assigned to a higher stage if this supplementary treatment had not been given. The retrospective estimate of tumour size was not sufficiently accurate (GARCIA et coll. 1963). Another difficulty is that the outcome may have been dependent on the surgical technique although this could not be established owing to the extremely low cure rate for the patients treated by surgery alone, and the small number of these patients.

Certain factors that may have had a bearing on the cure rate underwent a change during the various periods. The difference in the stage distribution between the periods suggests earlier diagnosis of the disease and possibly also more effective operations by more highly skilled surgeons, since patients with tumours bursting during the operation, that is stage III tumours, were fewer in the last period than in the two earlier ones.

Relatively little difference in the cure rates was evident for stages I and II, though 8 of the latter tumours invaded blood vessels, including the inferior vena cava. On the other hand, tumours involving the regional lymph nodes were assigned to stage III (21 patients), for which the cure rate was much lower. This investigation would appear to suggest that invasion of the regional lymph nodes, rather than of the inferior vena cava, is a factor of major prognostic significance.

The value of combined radiation therapy and surgical treatment is confirmed by the present findings. The improvement in the cure rate resulting from the supplementary preoperative irradiation was greater for this series than any others published (HARVEY 1950, KLAPPROTH 1959, KOOP et coll. 1964). Several authors (GROSS 1953, LADD & WHITE 1941, VAETH & LEVITT 1963) have expressed their reluctance to administer preoperative irradiation, mainly because of uncertainty regarding the diagnosis. With improved radiologic methods applied by experienced physicians the proportion of incorrect positive diagnoses has been reduced to about one per cent (SCHWEISGUTH).

From the overall results for the patients treated with actinomycin D it would appear that the addition of this drug therapy considerably raised the cure rate. When, however, the interaction of concomitant factors is taken into account this beneficial effect is no longer apparent. The patients given actinomycin D were

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The stage of the disease on admission was found to be of primary importance The addition of radiation therapy to surgery improved the results in stages I and II and such treatment before operation the results in stage III

ZUSAMMENFASSUNG

Eine retrospektive statistische Analyse mittels der automatischen Interaktions Erkenntnis Methode wurde zuzätzlich zu der einfachen tabularen Statistik bei 335 Patienten mit einem Nephroblastom, die während der Jahre 1927—1967 in skandinavischen Krankenhausern behandelt worden waren, vorgenommen um die Faktoren festzustellen, die fur die Prognose von Bedeutung sind Es fand sich, dass das Stadium der Erkrankung bei der Aufnahme von erstrangiger Bedeutung war Die zusätzliche Strahlentherapie zur Chirurgie verbesserte die Ergebnisse bei den Stadien I und II, und eine derartige Behandlung vor der Operation die Ergebnisse beim Stadium III

RÉSUMÉ

Pour évaluer les facteurs qui influent sur le pronostic *les* auteurs ont *fait une analyse* statistique rétrospective utilisant, en plus des simples statistiques tabulaires, la méthode de détection automatique d'interaction sur 335 malades atteints de néphroblastome et traités dans *les hôpitaux* scandinaves *de 1927 à 1967* Ils ont constaté que *le* stade de cette *affection* au moment de l'admission a une importance primordiale L'association d'un traitement par les radiations à la chirurgie améliore les resultats aux stades I et II, le traitement par les radiations fait avant l'operation améliore les résultats au stade III

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FROM THE SAFETY AND OPERATION DEPARTMENT, AB ATOMENERGI, STUDSVIK, 611 01 NYKOPING AND THE DEPARTMENTS OF RADIATION PHYSICS (DIRECTOR PROF G HETTINGER), AND RADIATION THERAPY (DIRECTOR PROF L.-G LARSSON), UMEÅ UNIVERSITY, 901 85 UMEÅ, SWEDEN

A ^{90}Sr—^{90}Y APPLICATOR FOR EXTRACORPOREAL BLOOD IRRADIATION

A HOLMER, M ERICSON, H SVENSSON and P WESTLING

Extracorporeal irradiation of the blood has been used in many centres since about 1965 for immunosuppressive purposes in renal transplantations (MERRIL et coll 1966, WOLF et coll 1966, HUME & WOLF 1967, ROSENGREN et coll 1968, PERSSON et coll 1969, ROSENGREN & SKÖLDBORN 1968) It has also been applied with positive results in some cases of leukemia (THOMAS et coll 1965, CRONKITE 1967)

The immunosuppressive effect probably depends on diminution of the number of circulating lymphocytes These cells, which are the most radiation sensitive of the blood cells, carry the antibodies responsible for the rejection reaction In renal transplantation cases extracorporeal irradiation of the blood has been used mainly in the treatment of rejection episodes as an adjunct to other immunosuppressive measures Good effects of such irradiation before transplantation have however also been reported

Submitted for publication 3 February 1972

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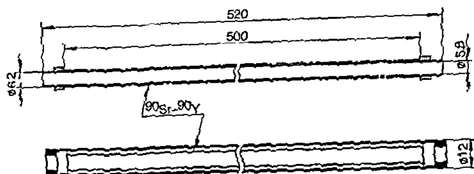


Fig 2 One of the four aluminium tubes with ^{90}Sr-^{90}Y coatings between support rings (above) Source assembly sealed with outer aluminium tube (below)

The external surface of the aluminium tubes between the two support rings (Fig 2) was subjected to an etching process in order to obtain a well adhering layer of ^{90}Sr-^{90}Y

An amount of 15 ml of the radioactive solution was mixed with ethyl alcohol and electrolyzed between platinum foil as anode and one of the aluminium tubes as cathode at 25 V until the desired yield was obtained, the tube was revolved during the electrolytic process Under the chosen conditions the cathode surface is not completely inert, an adhering crystalline layer is formed, identified as $Sr_3[Al(OH)_6]_2$ A ^{90}Sr deposit of about 12 Ci was obtained on each of the four tubes, corresponding to a coating thickness of 2 0 mg Sr/cm^2 Each of the aluminium tubes was surrounded by an outer aluminium tube in order to assure safe enclosure of the radioactive layer (Fig 2) The outer tube was sealed to the inner tube with glue, the sealed sources were then carefully tested for contamination and leakage and mounted in the radioactive shielding (Fig 3)

Operation A sterilized silicone rubber tube (ID 4 mm, OD 5 5 mm) is threaded into the irradiation channels by means of a lucite (plexiglas) bar fastened to one end The tube must be free from any trace of foreign matter, which might give rise to corrosion or wear of the aluminium irradiation tubes The integrity of the channels is controlled as a routine by making smear tests of the silicone rubber tube surfaces for β activity each time a tube is removed

The irradiator is placed alongside the patient at a distance of about one meter which ensures an exposure rate of under 10 mR/h One, two, three or four channels are used simultaneously, depending on the desired absorbed dose rate The blood is fed through the rubber tube at a rate of about 100 ml/min with an external pump The four channels together contain 25 ml blood in the irradiation zone

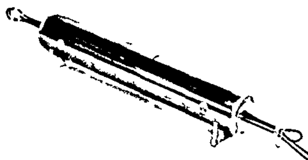


Fig 1 General view of the ^{90}Sr-^{90}Y blood irradiator

Extracorporeal irradiation of the blood has been employed in our centre since 1968 in 7 patients with renal transplants by the ^{60}Co technique described by ROSENGREN & SKOLDBORN (1968) These patients were later subjected to operation Some positive effects were observed and no complications attributable to the irradiation occurred The series, however, is obviously too small for evaluation

The ^{60}Co technique, which makes use of the irradiation units intended for ordinary radiation treatments, although simple, has several drawbacks of practical significance The irradiations require much shielding and long plastic tubes for conducting the blood past the source Furthermore, each treatment prevents the normal use of the irradiation unit for several hours

A ^{90}Sr-^{90}Y irradiator without these drawbacks will be described It enables the same or even higher mean absorbed dose rates for blood as obtained by the ^{60}Co source of 5 000 Ci to be achieved

A general view of the ^{90}Sr-^{90}Y blood irradiator appears in Fig 1 The radioactive source consists of four thin-walled aluminium tubes (ID 5 8 mm, OD 6 2 mm) covered on the outside with ^{90}Sr-^{90}Y (Fig 2) Each radioactive layer is first hermetically sealed by an outer aluminium tube and then all four tubes are mounted in a stainless steel jacket filled with lead as shielding material Both ends of the irradiation unit are provided with protective shields of a transparent plastic material thick enough to protect against the β-radiation The total length of the unit is 740 mm and the weight 27 kg

Preparation of the source One hundred Ci of ^{90}Sr in the form of strontium oxide powder were dissolved in hydrochloric acid and diluted with water to a volume of 100 ml The specific activity of the solution was 60 Ci ^{90}Sr/g Sr which is about 40 per cent of the theoretical value for pure ^{90}Sr

part of the tube, where the absorbed dose rate is lowest, than in the vicinity of the tube walls, where the absorbed dose rate is highest Thus the maximum mean absorbed dose rate for blood cannot be above the 6 per cent calculated for the flowing dosimeter solution

The rheologic difference between blood and $FeSO_4$ dosimeter solution for the flow geometry was investigated by KJELLSTROM (1971) He reported that the flow is laminar for both fluids and that this type of flow prevails in the major part of the tube channels There is a parabolic velocity distribution in both fluids, the velocity for blood is slightly higher in the wall region and about 5 per cent lower in the centre region than for the dosimeter solution

These differences in distribution of flow velocities result in a mean absorbed dose rate for flowing blood which is only a few per mille higher than that of the $FeSO_4$ dosimeter solution The 6 per cent higher mean absorbed dose rate evident in the stationary condition was confirmed in these calculations

The differences between the mean absorbed doses received by the dosimeter solution and blood flowing under identical conditions through the tube channels is negligible for the clinical use now being considered

Conclusion

The aim of the present work to construct and evaluate a simple irradiator giving about the same mean absorbed dose rates to the total blood volumes as a ^{60}Co irradiation unit used previously has been achieved The applicator was ready for clinical use in February 1971 and up to December 1971, 41 irradiations had been carried out for the purpose of immunosuppression before and after kidney transplantation without complications If future empirical or theoretic considerations would lead to a necessity to vary mean absorbed dose rates over a wide range this can easily be accomodated by the present irradiator and technique The number of channels used simultaneously during one treatment can be varied and channels of higher or lower source strength can be supplied

SUMMARY

A simple ^{90}Sr ^{90}Y applicator for the extracorporeal irradiation of the blood is described The irradiator allows mean absorbed dose rates to a total blood volume of 4 litres of up to 1 650 rad/h to be attained

ZUSAMMENFASSUNG

Ein einfacher ^{90}Sr ^{90}Y Applikator fur die extrakorporale Bestrahlung von Blut wird beschrieben Die Bestrahlungsanordnung ermoglicht es, mittlere absorbierte Dosisraten bis zu 1 650 rad/h bei einem Gesamtblutvolumen von 4 Litern zu erhalten.

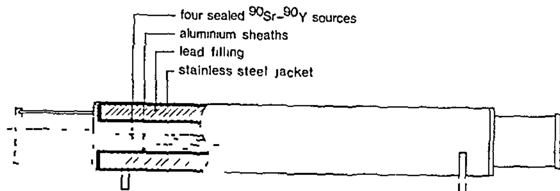


Fig 3 Schematic section of irradiation unit

The absorbed dose is assumed to be about the same for human blood and for $FeSO_4$ dosimeter solution under irradiation in the ^{90}Sr-^{90}Y irradiator The $FeSO_4$ dosimeter technique applied has been described by PETTERSSON & HETTINGER (1967), a G-value of 0 155 eV $^{-1}$ was used for calculations

Dosimeter solution was pumped through the rubber tube in the same manner as the blood at a flow rate of between 100 and 200 ml/min The four different channels gave mean absorbed dose rates between 3 900 and 5 000 rad/min and an average value of 4 380 rad/min The variation of source strength is explained by differences in the amount of ^{90}Sr-^{90}Y deposited on each individual aluminium tube channel As an example, if four channels be used and the total blood volume of a patient is 4 litres the mean absorbed dose rate to the blood is calculated to be 1 650 rad/h

The absorbed dose rate diminishes with the source distance from the inner tube wall towards the centre of the tube This was demonstrated by measurements with small glass dosimeters (diameter 1 mm), placed in a polystyrene rod having the same outer diameter as the silicone rubber tube One dosimeter was sited to correspond to the position of blood at the inner wall and the other to blood at the centre of the tube The centre absorbed dose rate was found to be 70 per cent of the value at the interior wall This figure is about the same as can be deduced for a corresponding geometry from theoretic curves given by SLATKIN & ROBERTSON (1970)

Measurements were also made with the dosimeter solution stationary in the tube channels This condition corresponds to that of the absorbed dose when blood is flowing at the same rate at any point of a section across the moving fluid column The mean absorbed dose rate worked out to be 6 per cent higher in the stationary condition than for the flowing solution The difference may be explained by the fact that the liquid is flowing at a higher rate through the central

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DETERMINATION OF SMALL MASS DIFFERENCES IN ROENTGENOGRAPHY

III Experimental investigations with a dental roentgen apparatus

GUNNAR LYSELL

The first paper in this series (LYSELL et coll 1968) concerned a theoretic analysis of the relation between the thickness of a given mass and the photographic density of its roentgen image and equations for this relation. The second paper (HOLLENDER & LYSELL 1972) dealt with the experimental determination of the thickness of objects by means of a roentgen diffraction apparatus and the above mentioned equations, first under nearly ideal conditions and secondly under conditions approaching those prevailing in routine diagnostic work. 16 kVp radiation and 54 kVp radiation were used. Narrow beam geometry was applied. At 16 kVp the radiation was practically monoenergetic, at 54 kVp, polyenergetic. The scattered radiation delivered to the films from the objects was reduced to a negligible amount by the use of a long object — film distance. The results indicated that the resolving power of the method was good, even at 54 kVp radiation. This means, among other things, that though polyenergetic radiation was used, values for the linear attenuation coefficient could be derived

Submitted for publication 18 August 1972

RÉSUMÉ

Description d'un applicateur simple pour l'irradiation extracorporeale par ^{90}Sr-^{90}Y du sang. Cet irradiateur permet d'atteindre des taux de dose moyenne absorbée pour un volume sanguin total de 4 litres atteignant 1 650 rad/h.

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Table 1

Linear attenuation coefficients for aluminium at 60 kVp radiation

Thickness of absorber (mm Al)	μ_{Al} at 60 kVp (cm^{-1})
0	5 36
0 05	5 32
0 15	5 21
1	4 40
2	3 64
3	3 09
4	2 80
5	2 67
5 1	2 66
5 3	2 65
6	2 63

the tubular diaphragm — mounted on the roentgen apparatus — to the optical bench With the film in the rearmost position the FFD was 440 mm and the OFD 110 mm, in the anterior position the film was 10 mm from the object The objects covered the upper part of the slit opening of the film holding device In the additional experiments the entire slit was covered by 5 05 mm aluminium situated in front of and in close contact with the objects

Determination of linear attenuation coefficients The transmission of the radiation from the dental roentgen apparatus through aluminium of different thicknesses was determined with the aid of two ionization chambers The logarithm of the transmission in per cent was plotted against aluminium thickness Triple determinations were made of the linear attenuation coefficients from the curve

Determination of the constant a The value of the constant a in the equation used was equal to the inclination of the straight line obtained when the logarithm of the photographic density values were plotted against the logarithm of the exposure values The determination was based on three characteristic curves

Variation in radiation intensity along the slit opening occurred in part II The focus of a dental roentgen apparatus is generally large It was therefore of interest to find out the magnitude of the spatial variation in intensity along the slit opening Six films were exposed at three different photographic densities The procedure was the same as in part II

with a fair degree of accuracy But the radiation from the roentgen apparatus was transmitted through a 5 97 mm aluminium filter at 54 kVp The linear attenuation coefficient therefore changed but little on introduction of extra absorbers of aluminium Furthermore, the diffraction apparatus had a constant voltage transformer for the voltage from the mains

In diagnostic dental roentgenography polyenergetic radiation is used, as a rule filtered through the equivalent of 2 mm aluminium Generally, the mains voltage is not controlled by a constant voltage transformer Furthermore, the film is close to the object with the result that scattered radiation to the film cannot be avoided

The present investigation concerns the determination of small mass differences, both with and without the influence of scattered radiation, using an ordinary dental roentgen apparatus

Material and Methods

In part II of this series two equations were used As they were found to be equally accurate, only one of them was applied, namely

$$d_2 = \frac{D_2 - D_1}{aD\,(\mu_1 - \mu_2)}$$

where d_2 denotes the thickness of the object (or thickness difference), D the photographic density, a a constant, and μ the linear attenuation coefficient A Ritter Century dental roentgen apparatus, operated at 60 kVp and 10 mA, was used The radiation had a half value layer of 1 45 mm aluminium The primary beam was restricted by aid of a tubular diaphragm (LYSELL 1957) with an inner diameter of 20 mm

The objects consisted of 108 5 μm thick aluminium foils of the same kind as earlier used (HOLLENDER & LYSELL) The foils were used in a single layer and in three layers, respectively Additional experiments were performed, where 5 05 mm aluminium of the same purity was added In this way mass differences of 108 5 and 325 5 μm, respectively, within an object could be secured

Film processing and densitometry Kodak periapical radia tized dental roentgen films were used The same standardized darkroom and densitometric procedures were used as in part II of this series

Irradiation geometry An optical bench and the rider with the device for film holding, described in part II, was used In addition, a second rider fixed

Table 4

Determination of foil thickness from photographic density measurements 60 kVp radiation The whole slit opening covered by 5 05 mm aluminium and in addition upper section covered by a 108 5 μm aluminium foil Distribution of calculated thicknesses at different levels of photographic density Two different object film distances

Thickness range (μm)	Mean photographic density above base and fog	
	Film in posterior position 0 412	Film in anterior position 0 443
91 – 100		2
101 110		
111 120		4
121 – 130	5	1
131 – 140	3	
141 – 150	2	
151 – 160		1
161 170		2
n	10	10
M	133 7	126 7
s	9 6	27 2
sM	3 06	8 59

Results

The linear coefficients are given in Table 1 Repeated measurement gave a mean value of 0 73 (range 0 72 — 0 74) for the constant α

Photographic determinations of foil thickness In the first part of the present investigation, *series of 10 films*, in one series only 9, were exposed, 3 series for each object thickness Exposure times were varied to secure 3 levels of photographic density The foil thickness was calculated from the above equation The results are given in Tables 2 and 3 The OFD was 110 mm The density differences were of the order of 0 01 — 0 15 units

In 4 series the mean values exceeded the 'true' ones The coefficient of variation varied from about 5 to about 12 per cent in the series with the thinner absorber With the thicker absorber the coefficient of variation never exceeded 5 per cent

In the second part of the present investigation the whole slit opening was covered by 5 05 mm aluminium in close contact with the objects, 108 5 μm and 325 5 μm aluminium respectively, covering the upper part of the slit Each combination was exposed in 2 series of 10 exposures each In the first series

Table 2

Determination of foil thickness from photographic density measurements 60 kVp radiation Upper section of slit opening covered by a 108 5 μm aluminum foil Distribution of calculated thicknesses at different levels of photographic density

Thickness range (μm)	Mean photographic density above base and fog		
	0 355	1 053	1 282
191—100			2
101—110	4	3	2
111—120	1	6	2
121—130	3	1	3
131—140	2		1
n	10	10	10
M	120 5	113 3	114 6
s	11 6	5 4	13 3
s_M	3 66	1 71	4 20

Table 3

Determination of foil thickness from photographic density measurements 60 kVp radiation Upper section of slit opening covered by 325 5 μm aluminium Distribution of calculated thicknesses at different levels of photographic density

Thickness range (μm)	Mean photographic density above base and fog		
	0 339	1 045	1 278
301—310		2	2
311—320	2	2	7
321—330		2	1
331—340		3	
341—350	5		
351—360	2		
361—370	1		
n	10	9	10
M	344 0	320 4	314 7
	15 1	11 1	4 0
s_M	4 78	3 68	1 27

Table 6

Variation in photographic density along the image of the slit opening uncovered by foils 60 kVp radiation Three different exposure times, 2 films at each Base and fog included

Film No	1	2	3	4	5	6
Section						
a	0 450	0 452	1 153	1 131	1 398	1 396
b	0 459	0 460	1 161	1 157	1 435	1 439
c	0 460	0 460	1 168	1 172	1 445	1 450
d	0 463	0 461	1 174	1 181	1 450	1 453
e	0 466	0 462	1 178	1 183	1 463	1 459
f	0 464	0 465	1 179	1 189	1 468	1 466
g	0 465	0 465	1 172	1 188	1 462	1 462
h	0 461	0 457	1 164	1 182	1 460	1 450

Table 7

Calculated mean foil thicknesses at various levels of photographic density with upper and lower section of the slit opening respectively, covered by 108 5 μm aluminium 60 kVp radiation

Mean photographic density above base and fog	Foil thickness (μm)		
	Upper section covered	Lower section covered	Total mean
0 318	111 6		
0 327		102 3	107 0
0 998	103 7		
1 092		90 5	97 1
1 145	86 4		
1 140		93 6	90 0
Total mean	100 6	95 5	98 0

	Upper section covered			Lower section covered		
n	5	5	5	5	5	5
M	111 6	103 7	86 4	102 3	90 5	93 6
s	12 6	5 2	5 7	14 0	16 2	5 0
sM	5 63	2 34	2 51	6 25	7 25	2 22

Table 5

Determination of foil thickness from photographic density measurements 60 kVp radiation The whole slit opening covered by 5 05 mm aluminium and in addition upper section covered by 325 5 µm aluminium Distribution of calculated thicknesses at different levels of photographic density Two different object film distances

Thickness range (µm)	Mean photographic density above base and f g	
	Film in posterior position 0 111	Film in anterior position 0 439
301–310		1
311–320		
321–330		2
331–340		3
341–350		3
351–360		1
361–370	1	
371–380	6	
381–390	2	
391–400		
401–410		
411–420		
421–430		
431–440	1	
n	10	10
M	382 3	336 5
s	18 9	14 6
sM	5 9[illegible]	4 60

the films were placed in the rearmost position, FFD = 440 mm and OFD = 110 mm, in the second series in the anterior position, FFD = 340 mm and OFD = 10 mm, respectively The exposure times were chosen to give approximately the same photographic densities in the 2 series The results are given in Tables 4 and 5 All mean values were above the 'true' level The coefficient of variation varied from less than 5 to about 21 per cent With films in the anterior position the calculated thickness values were generally lower than with films in posterior position, indicating an effect of scattered radiation The mean values were reduced by about 5 to 12 per cent

Variation in radiation intensity along the slit opening The results are given in Table 6 In areas used for densitometric measurements, the photographic density of the film was less in the upper section than in the lower one The differences were, however, very small

of scattered radiation could therefore be determined also under conditions similar to those, recommended for diagnosis of dental caries Scattered radiation had, with few exceptions a substantial effect on the thickness values

The image of the slit uncovered by foils demonstrated only a slight variation in radiation intensity in areas of interest for densitometric measurements This was much less marked than for the roentgen diffraction apparatus in part II Different radiation geometry offers one possible explanation With the roentgen diffraction apparatus the FFD was large Hence, the influence of extrafocal radiation was small With the dental roentgen apparatus the FFD was relatively small The primary diaphragm was cylindrical, without further restriction of the beam towards the focus Thus, even if the primary beam was limited, there was an influence of extrafocal radiation and this could to a certain extent level out the radiation intensity differences Another explanation might be that the slit opening was oriented perpendicular to the direction along which the spatial variation in intensity is large As all images of the slit opening uncovered by foils gave higher though only slightly higher photographic density values for the lower part than for the upper part, it was considered of interest to make calculations of foil thicknesses with respectively, the upper and lower section covered The results were compatible with the small fluctuation of intensity in that the differences were small

While most of the calculated values of foil thicknesses given in Tables 2 to 5 were above the true level the values in Table 7 were mostly below, although the upper section of the slit opening was covered in half of the determinations This could hardly be due to a systematic error in the determination of the linear attenuation coefficients Random variation of this magnitude could, nevertheless be caused by changes in mains voltage However, it should be noted that films of the same emulsion number were used for the determinations given in Tables 2 to 5 while films of another emulsion number were employed for the determinations given in Table 7 The influence of variation in film emulsion was not investigated In part II it was shown, however, that different emulsion numbers did not influence the general shape of the characteristic curve and thus did not change the a value

To summarize, with the thinner absorber, most thickness determinations were less than 10 μm from the 'true values The coefficient of variation varied from about 5 to about 12 per cent With the thicker absorber the corresponding values were about 14 μm and under 5 per cent, respectively When 5 05 mm aluminium was added most thickness determinations of the thinner absorber were less than 18 μm from the true value The corresponding value for the thicker absorber was 45 μm The coefficient of variation varied from less than 5 to about 21 per cent. No correction was made for spatial variation in radiation intensity along the slit opening

In order to examine the possible effect of this variation in intensity of radiation along the slit opening on thickness determinations, 6 series of 5 films each were exposed with a 108.5 μm thick aluminium foil, in 3 series, covering the upper section of the slit opening, and in 3 series covering the lower section. The results are given in Table 7. In 5 of the 6 series the mean values were below the 'true' level and in one, the mean value was lower for the upper section than that for the lower section. The coefficient of variation varied from about 5 to about 18 per cent.

Discussion

The degree of accuracy and precision of determination of foil thicknesses was similar to that found in part II with 54 kVp radiation. It should be noted, however, that in some respects the technical factors differed conspicuously.

The experiments were performed with a radiation quality used in dental practice. The source of radiation was an ordinary dental roentgen apparatus. In contrast with the diffraction apparatus earlier used there was no constant voltage transformer for voltage of the mains. Measurements performed showed that mains voltage fluctuated about ± 5 per cent. For instance a change in tube tension of about 5 per cent will give a variation of μ of about the same percentage. Thus, variation in radiation quality might have influenced the calculated values. In addition, the radiation was only slightly filtered. Its spectrum was therefore broad.

The change in linear attenuation coefficient with increasing absorber thickness (Table 1) was much more rapid in this investigation than in the earlier (HOLLENDER & LYSELL). The graphical representation might therefore be less accurate in this part. The transmission of the polyenergetic radiation through different layers of aluminium was registered with ionization chambers. The logarithm of the transmission in per cent was plotted against aluminium thickness. At any thickness of aluminium the slope of the resulting curve is a measure of the effective linear attenuation coefficient for the radiation energy transmitted through that aluminium thickness. In calculating object thickness (d_x) the linear attenuation coefficient was obtained at a point of the transmission curve corresponding to an aluminium thickness of $\frac{d_x}{2}$, i.e. 0.05 and 0.15 mm aluminium for the thinner and the thicker absorber, respectively. When 5.05 mm aluminium was added, the corresponding values were 5.1 and 5.2, respectively. High frequency fluctuation of mains voltage will influence precision. Low frequency changes could systematically affect the determinations.

In the second part of the experiments the whole slit opening was covered with 5.05 mm thick aluminium in close contact with the objects. The influence

RÉSUMÉ

Des experiences faites avec un appareil de radiographie dentaire ont montre que dans certaines conditions de petites differences de masse dans un objet peuvent etre determinees d'après les mesures de densite photographique au moyen d'un equation etablie dans les conditions d'un rayonnement mono-energetique

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The results obtained show that it is possible to determine the thickness of objects, *consisting of 108.5 and 325.5 μm thick aluminum foils* with good accuracy and precision, most determinations being about 10 to 20 per cent from the 'true' values. In comparison, in clinical work a caries lesion less than *0.5 mm in depth is of little interest. Thus, the resolving power of the method* must be considered good. This investigation furnished some valuable information on the clinical adaptation of the method.

A power supply for constant mains voltage should be of great value. The primary, tubular diaphragm minimized the scattered radiation. In combination with filmholder an ideal projection could be arranged and a reproducible *irradiation geometry could* be obtained. *Thus, it will* be possible not only to determine a small mass difference within an object in vivo, but also to control quantitatively the healing processes, to follow up the progress of carious lesions, or to determine the effect of prophylactic agents.

In this investigation μ_2 denotes the linear attenuation coefficient for air and is considered negligible compared to μ_1 (linear attenuation coefficient for Al) in the calculations. In clinical examinations this would not have been correct. For instance, if a carious lesion was examined, μ_2 denotes the linear attenuation coefficient for soft tissue and has to be determined experimentally for the actual radiation spectrum.

It was pointed out in part II that measurements should be based on several independent determinations. In a clinical investigation this requirement cannot be *fulfilled because of the radiation hazards. This implies that repeated determinations* of the same thickness difference on different occasions could differ, though rarely, by as much as 70 μm aluminium. Follow-up examinations of the same object *will, on the other hand, probably increase the discrimination* level of a change in thickness or mass.

SUMMARY

Experiments performed with an apparatus for dental roentgenography demonstrated that *under certain conditions* small mass differences within an object could be determined from photographic density measurements with an equation derived under conditions of monoenergetic radiation.

ZUSAMMENFASSUNG

Experimente, die mit einem Dental-Röntgenapparat vorgenommen wurden, zeigten, dass unter gewissen Bedingungen *kleine Massenunterschiede innerhalb eines Objektes aus photo*graphischen Dichtemessungen unter Anwendung einer Gleichung, die unter den Bedingungen monoenergetischer Strahlung hergeleitet worden war, bestimmt werden konnten.

Little information is available, however, on this subject in semiconductor devices used as dosimeters in the dc mode of operation Moreover the observations of various workers on temperature effect are not in agreement

With detectors run in the short circuit configuration, sensitivity to temperature was reported to be either completely zero (GULBRANDSEN & MADSEN 1962, SCHARF 1960) or of negligible magnitude (PARKER & JOHNSON 1969), whilst changes in the output current were observed in SCHARF's later work (1966) and on a large number of surface barrier coaxial probes by ALLSWORTH (personal communication)

In the work being reported here the response of the semiconductor detectors was investigated in the temperature range of 10° — 40 °C, being of interest in clinical dosimetry Higher temperatures were not used to avoid damage to the devices which may occur due to thermal expansion of internal electrode connections and to avoid possible permanent deterioration of the detector performance Measurements were made on silicon p n junction surface barrier detectors of cylindrical geometry 6mm and 3mm in diameter, developed at AERE, Harwell (ABSON et coll 1968) for clinical use and on planar devices of general application Water bath of controlled temperature was utilized to vary the ambient temperature throughout these investigations The output current was measured by means of a specially developed picoampere level amplifier of low input impedance providing also the facility of variable load resistance for the detectors investigated In experiments involving radiation Cs 137 sources were used and care was taken to avoid any changes in scattering conditions

Output signal of the semiconductor detector

The theory of operation of p n junction detectors and the mechanism of radiation detection has been discussed by many workers (JONES 1963, SCHARF 1960 DEARNALEY & NORTHROP 1966, BERTOLINI & COCHE 1968) and will therefore be omitted However, some points helpful in the consideration of the measurement of the output signal and of the temperature effect will be reviewed in much simplified form

In the process of formation of a p-n junction a strong electric field is established in a *narrow transition zone* between the p type and n type layer, also called the depletion region, or space charge region The depletion region is present even when no external bias is applied across the junction The electric field is directed from the n type to p-type layer, so that electrons and holes produced by radiation in the depletion region (or outside it, but diffused into it) are driven to the n type or p-type material respectively Thus when the

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TEMPERATURE RESPONSE OF SILICON SURFACE BARRIER SEMICONDUCTOR DETECTORS OPERATED IN THE DC—SHORT CIRCUIT CONFIGURATION

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Semiconductor detectors are finding increasing application in medicine as nuclear detectors, spectrometers and dosemeters Their small size, with high sensitivity and ease of handling, render them specially well suited for clinical dosimetry in body cavities An example of such an application is the measurement of bladder and rectal doses during intracavitary irradiation of gynaecologic carcinoma

Several favourable reports have been published on the clinical experience gained in the use of these devices (JONES 1963, PARKER & JOHNSON 1969, PARKER 1970) *However, problems are encountered with the temperature* sensitivity of the p n junction semiconductor detectors when used in the clinical environment in which a detector is subjected to a sudden ambient temperature change, i e from room to body temperature

Temperature effects in semiconductor detectors used in spectroscopy have been intensively investigated and the technique is accepted of operation in stabilized ambient temperature

Submitted for publication 2 May 1972

(thermally generated carriers, edge and surface leakage effect) resulting from the biassing voltage

By operating the diode with no bias voltage, the leakage currents are reduced to picoampere level and lower radiation intensities can be detected In these conditions there is a close similarity in the behaviour of the p n junction detector to that of a photovoltaic light cell As with the latter, the radiation induced signal of the detector can be measured either in the open circuit configuration in terms of voltage signal, or in a short circuit configuration as the current induced in the outer circuit of the p n junction In both cases a relatively simple instrumentation is required for signal amplification

It can be shown that the short circuit mode offers linear dose rate detector current relation and is therefore more attractive than the voltaic configuration in which these quantities follow a more complex function

Short circuit mode of operation The electrical behaviour of a p n junction radiation detector operated in the short circuit mode can be deduced by considering the circuits in Fig 1, in which the detector is represented by a current generator (I_d) and its parallel connected internal resistance (R_j) I_d is the current generated in the detector flowing internally from n type layer to p type layer In Fig 1 a the terminals of the device are short circuited (the resistance of the external circuit $R_E = 0$) The whole of the detector current flows through the external circuit, in which it can be measured As $R_E = 0$, there is no voltage potential across the terminals ($V = 0$) and the short circuit conditions are fulfilled

It will be noted that the detector current I_d consists of two components, I_r = the radiation generated current and I_o = the current which flows through the p n junction even when no radiation is acting upon the detector and no external bias is applied (cf p 132) The latter will be further referred to as detector thermal current in order to distinguish it from the leakage or reverse current which is of similar origin but of much larger magnitude and flows under a completely different circuit arrangement

In order to measure the detector current, an instrument must be connected into the external circuit, having a finite internal resistance, R_E The circuit therefore takes the form shown in Fig 1 b The consequence of the presence of R_E is that the detector current now divides between R_j and R_E and therefore the current measured in the external circuit is smaller always by the fraction which flows into the internal resistance branch of the circuit

$$\text{Hence } I_E = I_d - I_j \text{ and } I_E = I_d \times \frac{R_j}{R_j + R_E} \tag{2}$$

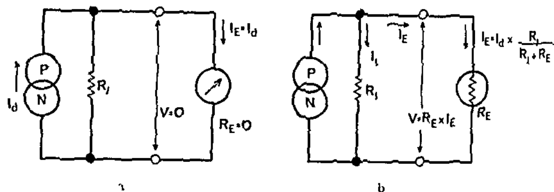


Fig. 1. Semiconductor detector connected in the short circuit, dc mode of operation, a) Pan ideal circuit. b) Practical circuit.

junction is irradiated with ionizing radiation a current is generated, flowing in the reverse direction in the detector.

According to SCHARF (1966) the radiation induced current in a detector of p-type base can be obtained by the simplified relation

$$I_R = eAg(w + L_n) / (\mu L_n + 1) \tag{1}$$

where e = electronic charge, A = irradiated silicon surface area, g = electron hole pair generation rate, w = width of the depletion region, μ = linear attenuation coefficient in silicon, L_n = diffusion length of electrons.

It is seen from eq. (1) that I_P is, for a given radiation, independent of crystal dimensions and mainly determined by the charge collection volume. I_R is proportional to the exposure rate, because of the dependence of the generation rate g on the exposure rate which is given by the relation

$$g = \frac{86.9\ \mu_{en}}{\xi\ (\mu_{en}/\varrho)\ \text{air}} \triangle X/\triangle t$$

where μ_{en} = linear energy absorption coefficient of silicon, (μ_{en}/ϱ) air = mass energy absorption coefficient in air, ξ = energy required for the production of electron-hole pair in silicon, $\triangle X/\triangle t$ = exposure rate.

From the point of view of the output signal measurement there are two distinct modes in which the semiconductor detector can be run, with an external reverse bias voltage and without an external bias.

With the external bias applied, charge pulses are collected at the detector terminals, amplified and registered by means of a scaling counter or count-rate meter. A discriminating device is used to eliminate noise and spurious signals. This technique has limitations in the level of the dose rate to be measured due to a considerable leakage current of the order of microampere

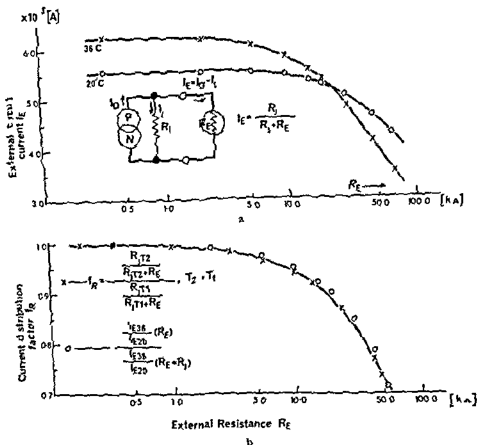


Fig. 3. a) Current in the external circuit at room and body temperature as a function of the external resistance. b) Comparison of the variation of the current distribution factor f_R with the measured variation of the external circuit current values at room and body temperature.

circuit used for measuring the current voltage characteristic of semiconductor diodes. The external bias applied was very small, of the order of a millivolt, to secure operation on the straight portion of the characteristic. Indeed, for such low bias voltages, the characteristic is completely linear in both positive and negative bias directions. The current flowing through the device in this condition was measured with a low input impedance picoammeter to avoid errors due to variable R_I/R_E ratio.

A strong dependence of R_I upon temperature was found (Fig. 2) in all the detectors investigated, R_I decreasing with rising temperature (silicon has

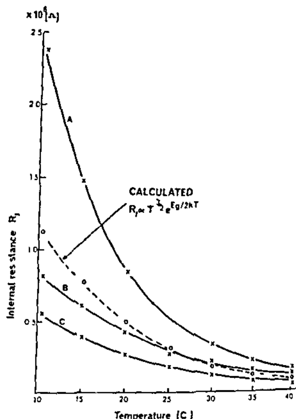


Fig 2 Variation of the p n junction internal resistance with temperature as found for several detectors The dotted line illustrates the variation of the silicon resistivity according to relation $\varrho \propto T^{3/2} \exp(Eg/2kT)$

It is seen from eq (2) that the magnitude of the external circuit current I_E follows the variation of $R_j/R_j + R_F$ with all other parameters fixed As R_F is taken smaller to approach zero value, I_E approaches I_d and the circuit goes into the ideal short circuit configuration Although in practice R_F is never zero, I_d can be determined by extrapolation of R_F towards its zero value

A close approximation to the ideal situation is obtained in practical circuit by making R_F small compared with R_j For example, if $R_j/R_E = 1\,000$, the current in the external circuit differs from the true detector current by 0 1 per cent, a discrepancy negligible for most practical purposes However, the internal resistance of semiconductor detectors does not remain constant with variable ambient temperature

Variation of the p-n junction resistance R_j with temperature

The investigation of the behaviour of the internal resistance of the p-n junction was carried out with the detector immersed in a water bath of controlled and adjustable temperature and with the detector connected in a

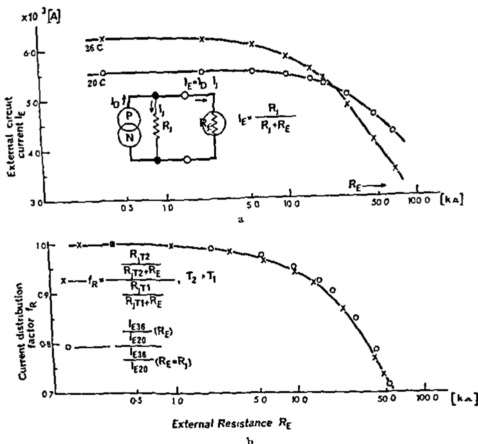


Fig 3 a) Current in the external circuit at room and body temperature as a function of the external resistance b) Comparison of the variation of the current distribution factor f_R with the measured variation of the external circuit current ratios at room and body temperature

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A strong dependence of R_J upon temperature was found (Fig 2) in all the detectors investigated, R_J decreasing with rising temperature (silicon has

negative temperature coefficient). Graphical analysis of the $R_{j(T)}$ curves revealed that the resistance change in the temperature range 10 — 25° C follows an exponential relation with the slope varying for individual detectors, being between 80 kΩ and 9 kΩ per degree centigrade. At higher temperatures, the $R_{j(T)}$ relation seems to obey a power law with an exponent of approximately —3/2. The transition from an exponential to a power relation is gradual and takes place between 25 and 30° C.

The theory of the semiconductor p-n junction diode suggests a purely exponential junction resistance-temperature relation. However, a semiconductor detector operated in a dc mode with a zero bias has the electrical characteristics of a temperature-sensitive resistor and it seems that the expression describing the temperature effect in a doped semi-conductor material may be more appropriate here. In this case the expression for the variation of resistivity with temperature comprises two terms of the type

$$R_j \sim T^{-3/2} \exp\,(Eg/2kT) \tag{3}$$

where Eg is the energy gap and k the Boltzmann constant.

Detailed comparison of measured values with R_j variations calculated by expression (3) gives good agreement, especially for higher temperatures for which the slope of the experimental curves and the calculated one are in accord.

The large separation between R_j values at low temperatures can be attributed to differences in the resistivity of the silicon used for the fabrication of these devices Surface currents can also be expected to have some influence, but their contribution could not be determined

In general, higher junction resistance values were found with the planar than with the cylindrical detectors.

It appears that R_j changes arise mainly from the effect of temperature on the bulk of silicon.

R_F— R_j interplay in the temperature effect. Fig. 3 shows the current measured in the external circuit of the detector, exposed to a dose rate of approximately 60 R/h, as a function of the resistance in the external circuit, R_T, and the ambient temperature It is of interest to consider the behaviour of the detector in more detail.

It is seen that at a given temperature, the current in the external circuit increases with decreasing R_E because the ratio R_F/R_j becomes smaller so that a greater proportion of the detector current flows into the external circuit (cf. p. 127). At arbitrarily small values of R_E, approximation to ideal short circuit conditions is obtained and $I_E = I_D$

The current curves taken at both room temperature and average body temperature show generally identical features However, it will be noted that at high values of R_E, smaller current was measured in the external circuit at 36° C than at room temperature This is due to the fact that the internal p n junction resistance is smaller at the higher temperature The ratio of resistances $R_j/(R_j + R_E)$ is therefore smaller at 36° C than at 20° C for the same value of R_E, resulting in the flow of the detector current I_D more into the R_j branch of the circuit With arbitrarily small R_E, the variations of the p n junction resistance have no effect on the distribution of current $R_j/(R_j + R_E) \approx 1$ in this condition) and the observed higher value of the external circuit current at body temperature is entirely due to the thermal changes in the detector current

It follows that the result of the change of detector temperature, as observed in the external circuit, depends on the interrelation between the change in the detector current, the p n junction resistance as well as the magnitude of R_E

The expected change of the current in the external circuit after the thermal quiescent state is reached can be estimated by means of a factor formed by the ratio

$$f_R = \frac{R_{jT_2}}{R_{jT_2} + R_E} \Bigg/ \frac{R_{jT_1}}{R_{jT_1} + R_E}$$

where R_{jT_1} and R_{jT_2} are the values of the internal resistance of the p n junction at considered temperatures T_2 being the higher

This factor, further referred to as the current distribution factor, is useful in the analysis of the temperature effect Its variation was numerically estimated for the detector of Fig 3 and compared with the experimentally determined ratios of the external circuit $(I_{E_{36}}/I_{E_{20}})$ at room and body temperatures These were normalized to the short circuit value and are shown in Fig 3 b A good agreement is seen between the predicted and the measured current variations, thus confirming the considered R_E—R_j interrelation

The two current curves seen in Fig 3 cross over at a certain value of R_E At the point of intersection a situation is reached at which the increase in the detector current $\triangle I_d$ is equal to the change of current distribution into R_j due to decrease in the latter In these circumstances the de[illegible] ears to be [illegible] resistance

[illegible] een the variation of the p n junction resistance and the external circuit resistance, in the devices exhibiting an increase of short circuit current can be used to advantage in order to stabilise the detector

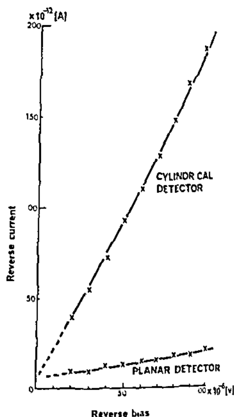


Fig 4 Surface barrier p n junction reverse current at ultra low bias voltages

response to temperature To accomplish this, the external resistance R_E should be made equal to the value indicated by the cross over point ($R_E = R_c$).

Detector thermal current I_0

All junction devices show currents even when not exposed to radiation These currents arise from several sources diffusion, space charge generation and surface The first contribution is due to the diffusion of the minority carriers from the field-free region into the depletion region It is a function of specific resistivity of the material, life time of minority carriers, diffusion length, mobility of minority carriers and carrier density

The second current is due to recombination centres in the space charge region Here the centres generate electrons and holes at a rate depending on the cross-section, density of such centres and their location in the forbidden energy gap It varies with the mobility, life time, the resistivity, and the width of the depleted region

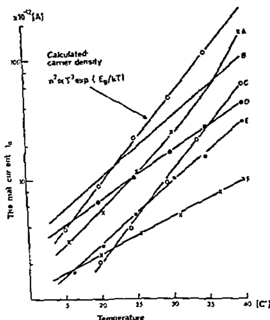


Fig 5 Dependence of the detector thermal current I_0 on the ambient temperature The dotted line illustrates the variation of the carrier density with temperature according to $n_i^2 \propto T^3 \exp(-Eg/kT)$

In general, the detector current is greater than the sum of the two components mentioned above, due to the surface current This current is the least predictable in the junction device, being subject to change as the surface chemistry at the edge of the junction varies with time, bias and temperature

Changes of temperature will introduce variations in the factors which influence the magnitude of these individual currents By far the most important of these changes is the increase in diffusion and space charge generation currents In each of these the carrier density n_i varies as $\exp(-Eg/2kT)$, (DEARNALEY & NORTHROP 1966, BERTOLINI & COCHE 1968) Carrier life time changes exponentially, also, through changes in n_i The dependence of the non radiation current on the exponent Eg/kT explains why semiconductors with narrow energy gap will never be satisfactory at room temperature

The physical origin and contribution of the various currents to the total reverse current have been investigated widely and a theory developed allowing prediction of their magnitude However the reverse current measured is often found one or two orders of magnitude higher than the one calculated (LANGMANN & MEYER 1966) In a biassed device the reverse current is of the order of 10^{-7} to $^{-8}$A, with the space charge generation current being the major contributor It decreases when the voltage bias is decreased approaching

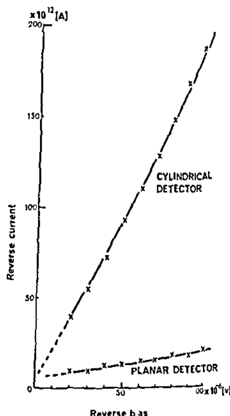


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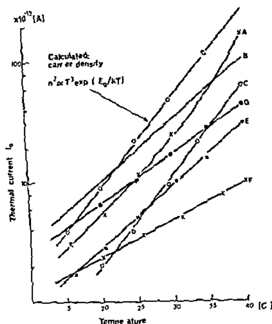


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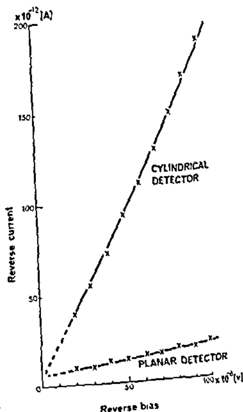


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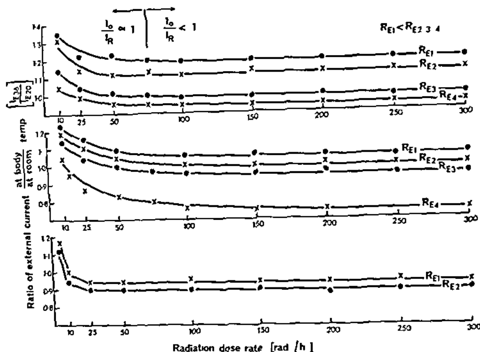


Fig 6 Temperature response curves illustrating the various patterns of behaviour as found with silicon surface barrier detectors $R_{E_1} = 0\,33$ kΩ $R_{E_4} = 100$ kΩ

current is probably smaller than the diffusion current contribution Considerable variability of I_0 with time at room temperature was observed which may perhaps be attributed to some surface effects The degree of sensitivity of I_0 was found to differ with individual detectors An attempt was made to correlate this with the basic characteristic of a p n junction, but without success

Temperature response curves

The investigations described in the previous sections provide some evidence that the temperature effect in a semi-conductor detector has several components, and the current change observed in an external circuit depends on all these factors and on their inter relation An experiment was therefore devised which allowed the component factors to come into play in such a way that their individual contributions could be determined Detectors were irradiated at

nanoampere at millivolt level At bias voltages below a millivolt, the current voltage function takes a linear relation of the type $I_0 = V_{bias}/R_j$, thus the slope is determined by the internal resistance of the detector at the given temperature

Fig 4 illustrates the behaviour of the reverse current at ultralow bias voltages as found for a planar and cylindrical device at room temperature in a circuit with $R_F \ll R_j$ With microvolts only applied to the p n junction the current amounts to approximately 10^{-10}A and shows considerable noise

The noise arises due to the dynamic nature of the charge balance across the p-n junction and statistical fluctuations in the number of carriers leading to changes in conductivity (shot noise). Thermal noise is generated as well, due to fluctuations in the spatial distribution of carriers arising from thermal diffusion Noise increases with temperature and its contribution becomes more significant with decreasing bias voltage (MASUDA & TAKEDA 1967) It manifests in the form of current fluctuations in amplitude and direction and because of its presence the determination of the current value below 20μV of bias voltage becomes uncertain

Without an external bias applied, thermal generation of carriers is responsible for the detector current Its magnitude is small, of the order of 10^{-11}A, with considerable noise superimposed ($\pm$ 5pA) Similar values of the thermal current I_0 were observed in all of the devices investigated

It is, however, not the magnitude of the I_0 current that matters but its variation with the ambient temperature This correlation was investigated using a technique as follows

With the detector subjected to an arbitrarly weak exposure the ambient temperature was changed from 20° to 40° C After the time needed for the detector to reach thermal equilibrium the exposure was removed The value to which the detector current dropped in these circumstances was then determined with the device still at 40° C Possible drift of the measuring system was checked, and corrected, if necessary, by cooling the detector down and noting the process of returning to zero The experiments were recorded on a chart recorder and repeated for the temperatures of interest From the knowledge of the detector sensitivity and the exposure the mean value of this residual thermal current I_0 was estimated Fig 5 illustrates the results It is seen that the thermal current increases considerably with the temperature, and seems to follow the changes in the carrier density n_i^2 An increase of peak-to peak noise was also observed but its quantitative evaluation was not made

At this stage of the work being reported it was not possible to determine, nor to make distinction, in the contribution of the diffusion, generation and surface current to the thermal current I_0 However, in view of the lack of an external bias, hence small width of the depletion depth, the space charge generation

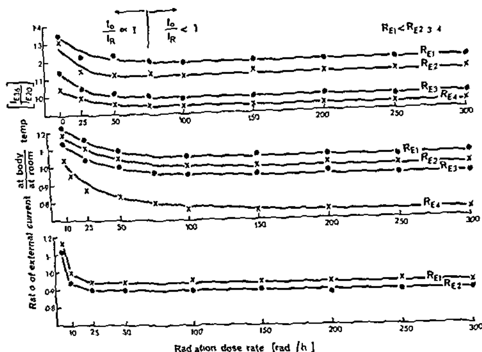


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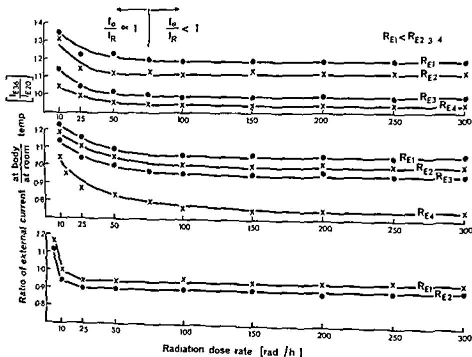


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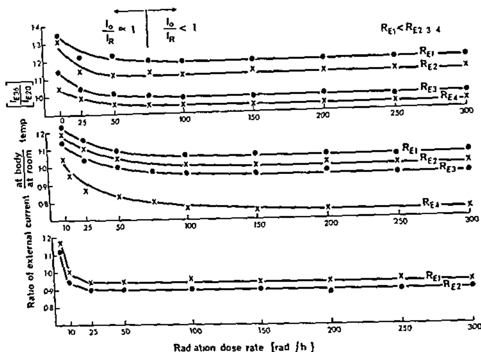


Fig 6 Temperature response curves illustrating the various patterns of behaviour as found with silicon surface barrier detectors $R_{E1} = 0\ 33\ \text{k}\Omega$ R_{E4} 100 kΩ

current is probably smaller than the diffusion current contribution Considerable variability of I_0 with time at room temperature was observed which may perhaps be attributed to some surface effects The degree of sensitivity of I_0 was found to differ with individual detectors An attempt was made to correlate this with the basic characteristic of a p n junction, but without success

Temperature response curves

The investigations described in the previous sections provide some evidence that the temperature effect in a semi conductor detector has several components, and the current change observed in an external circuit depends on all these factors and on their inter relation An experiment was therefore devised which allowed the component factors to come into play in such a way that their individual contributions could be determined Detectors were irradiated at

nanoampere at millivolt level At bias voltages below a millivolt, the current voltage function takes a linear relation of the type $I_0 = V_{bias}/R_j$, thus the slope is determined by the internal resistance of the detector at the given temperature

Fig 4 illustrates the behaviour of the reverse current at ultralow bias voltages as found for a planar and cylindrical device at room temperature in a circuit with $R_F \ll R_j$ With microvolts only applied to the p-n junction the current amounts to approximately 10^{-10}A and shows considerable noise

The noise arises due to the dynamic nature of the charge balance across the p-n junction and statistical fluctuations in the number of carriers leading to changes in conductivity (shot noise) Thermal noise is generated as well, due to fluctuations in the spatial distribution of carriers arising from thermal diffusion Noise increases with temperature and its contribution becomes more significant with decreasing bias voltage (MASUDA & TAKEDA 1967) It manifests in the form of current fluctuations in amplitude and direction and because of its presence the determination of the current value below 20μV of bias voltage becomes uncertain

Without an external bias applied, thermal generation of carriers is responsible for the detector current Its magnitude is small, of the order of 10^{-11}A, with considerable noise superimposed ($\pm$ 5pA) Similar values of the thermal current I_0 were observed in all of the devices investigated

It is, however, not the magnitude of the I_0 current that matters but its variation with the ambient temperature This correlation was investigated using a technique as follows

With the detector subjected to an arbitrarly weak exposure the ambient temperature was changed from 20° to 40° C After the time needed for the detector to reach thermal equilibrium the exposure was removed The value to which the detector current dropped in these circumstances was then determined with the device still at 40° C Possible drift of the measuring system was checked, and corrected, if necessary, by cooling the detector down and noting the process of returning to zero The experiments were recorded on a chart recorder and repeated for the temperatures of interest From the knowledge of the detector sensitivity and the exposure the mean value of this residual thermal current I_0 was estimated Fig 5 illustrates the results It is seen that the thermal current increases considerably with the temperature, and seems to follow the changes in the carrier density n_i^2 An increase of peak-to peak noise was also observed but its quantitative evaluation was not made

At this stage of the work being reported it was not possible to determine, nor to make distinction, in the contribution of the diffusion, generation and surface current to the thermal current I_0 However, in view of the lack of an external bias, hence small width of the depletion depth, the space charge generation

rates depends on the steady increase of I_R/I_0 with increasing exposure rate A detector which has thermal current inherently large in value and very sensitive to temperature would produce curves with larger slopes and for such a device a much larger exposure rate is required before the condition $I_R \gg I_0$ is reached

To summarize the relative response of a detector to temperature clearly has two domains, an $I_R/I_0 \approx 1$ region in which the thermal change in I_0 is important and the overall temperature effect is exposure rate dependent, and an $I_R/I_0 > 1$ region in which I_0 is insignificant and the temperature effect is independent of exposure rate In both domains R_E is important in determining the effective temperature coefficient

A family of temperature response curves is useful for the derivation of essential information about a particular detector The magnitude of the thermal current can be estimated from the data available at low exposures and the true temperature coefficient is clearly seen The judgment of the possibility of using a detector in a temperature compensated circuit can be made and the approximate value of the cross over resistance can be estimated The spacing between the particular curves taken at various R_E, being the function of the current distribution factor, gives the ability to assesss the internal resistance of the p n junction

Temperature response pattern of the radiation generated current I_R

The results shown in Fig 6 suggest the experimental conditions for studying the variation of the radiation generated current, I_R, in p n junction detectors with changes in the ambient temperature These conditions are that the radiation exposure rate should be large enough to render fluctuations of I_0 with temperature negligible, i e $I_R/I_0 \gg 1$, and that all of the current generated in the detector should flow into the external circuit, i e $R_E/R_j \ll 1$ An experimental determination of $\Delta I_{R(T)}$ was carried out under these conditions within the temperature range 10° to 36° C The results are shown in Fig 7 in terms of the ratios

$$I_{R(T_2)}/I_{R(20\ C)}$$

Data on several detectors are included to illustrate the variety of the response pattern observed in the devices investigated It is seen that both negative and positive current coefficients were observed in the room to body temperature range The $I_{R(T)}$ relation does not seem to follow any uniform pattern Seven out of ten devices have shown an increase of current between 2 and 23 per cent, (20° to 36° C), with three detectors, a negative response was noted

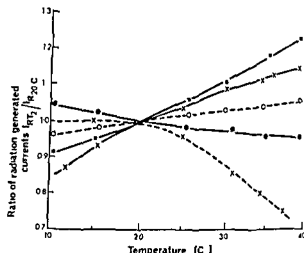


Fig 7 Relative changes of the radiation generated current I_R due to temperature as found with various detectors measured in a circuit with $R_j/R_E \gg 1$ and radiation exposure for which $I_R/I_0 \gg 1$

constant (but variable) exposure rate and, whilst under irradiation, subjected to a sudden change in temperature, from 20° to 36 6° C The external current I_E was measured at both temperatures and hence the ratio I_{E36}/I_{E20} found Measurements were carried out at various exposure rates and with values of external resistance R_E ranging from 0 33 kΩ to 100 kΩ The results, for three detectors, illustrating the possible type of response which a p-n junction may give are illustrated in Fig 6

The features of Fig 6 which require explanation are (1) at arbitrarily high exposure rates, the ratio I_{E36}/I_{E20} is almost independent of exposure rate and may be > or <1 0, (2) at low exposure rates the ratio is >1 0, i e the external current increases with increasing temperature and the effect is most marked at low value of R_E This is because the detector thermal current, I_0, is large compared with the radiation induced current I_R and its change adds to the radiation current changes Thus, I_{E36}/I_{E20} depends on the contribution of thermal change in $I_0 + I_R$ With increasing exposure rate, I_R/I_0 increases rapidly and the thermal change in I_0 becomes insignificant compared with the thermal change in I_R The curves indicate that the temperature coefficient of I_R is independent of the precise value of I_R and hence is independent of the exposure rate This has been found to be true of all the detectors tested in the present investigations The apparent negative temperature coefficient at high values of R_E (i e $I_{E36}/I_{E20} < 1$ 0) is compatible with the observations mentioned on page 128 and is attributable to the change in the current distribution factor f_R, with R_E and $R_{j(T)}$

The slight slope of the curves in Fig 6 in the medium range of exposure

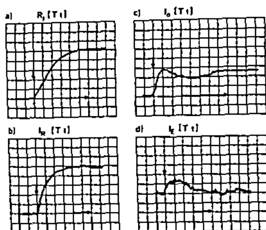


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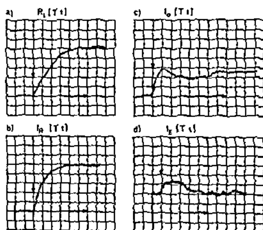


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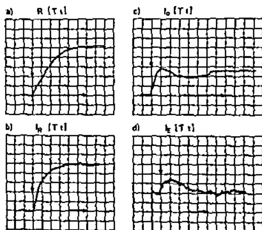


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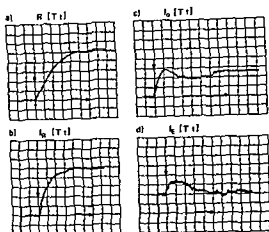


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From knowledge of the time constants and the magnitude of the quantities involved the thermal transient wave was constructed by graphical summation, and is shown in Fig 9 It is seen that the empirical transient is in very good agreement with the experimentally recorded curve providing proof for the above considerations

The transient curve was observed with all of the detectors which qualified for operation in a compensated circuit Differences in individual devices occur in the magnitude and duration of the current wave which can be explained by differences in sensitivities and masses of the detectors involved However, the time displacement between the temperature effect on I_R and R_j was observed in all the measured devices, both of planar and cylindrical geometry The amplitude of the transient is directly related to the detector temperature sensitivity

An additional contributor to the transient wave appears when a detector is used for detection of small exposures ($I_R/I_0 \approx 1$ domain) In this case, the contribution of the variation on the detectors thermal current I_e becomes apparent resulting in the enlargement of the transient wave Some of the detectors investigated have shown the presence of a very fast varying component (Fig 8) superimposed on the thermal current transient I_e which is attributed to the thermo electric e m f induced in the internal connections of the detector (ALLSWORTH, personal communication)

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In the course of the experiments carried out so far, three quantities R_j, I_e and I_R were found to contribute to the temperature effect observed in the external curcuit of a p n junction detector

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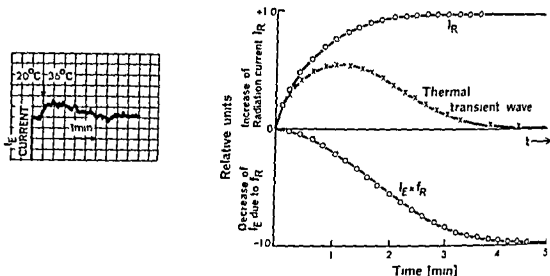


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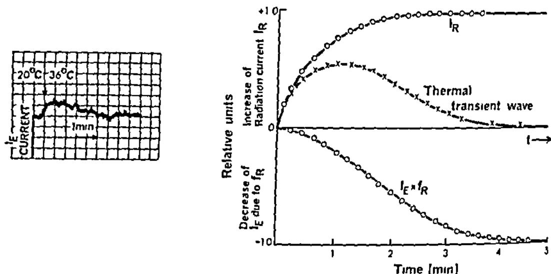


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kompensierten Schaltung vorgenommen Es wurde gefunden, dass Detektoren von sowohl planer als auch zylindrischer Geometrie ahnliche Temperatureigenschaften haben und es wurde gezeigt, dass drei Faktoren zum Temperatureffekt beitragen die Veranderungen in dem durch d e Strahlung hervorgerufenen Strom und dem Warmestrom und der internen Impedance des PN Übergangs Deren Verhalten und Beitrag wurden untersucht Die PN Übergangsdetektoren konnen sowohl positive als auch negative Temperatur Koeffizienten haben ein Effekt, der sich nicht vollständig durch die klare Theorie des PN Übergangs erklaren lasst Die Detektoren die einen Anstieg des Stroms mit steigender Temperatur zeigen sind fur die Arbeit in der kompensierten ausseren Schaltung gunstig und deshalb fur die Verwendung bei klinischen Messungen anwendbar

RÉSUMÉ

La réponse en fonction de la température de dispositifs à barrière de surface avec jonction p-n aux silicones utilisés comme dosimètre dans la technique de court-circuit de a été étudiée, l auteur a fait une analyse de leur fonctionnement dans un circuit a compensation de tempera ture Il a constaté que les détecteurs de forme plane et de forme cylindrique ont des caractéristiques de température similaires et il a montré qu il y a 3 facteurs de l effet de température les variations dans le courant produit par la radiation les modifications dans le courant thermique et l impedance interne de la jonction p-n Il a étudié leur comportement et leur importance Les détecteurs à jonction p-n peuvent avoir un coefficient de température positif ou négatif cet effet n est pas très clairement explicable par la théorie de la jonction p-n Les détecteurs qui presentent une augmentation du courant quand la température augmente sont adaptés au fonctionnement dans le circuit externe compensé et sont pour cette raison utiles pour l application dans les mesures cliniques

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Thus the response measurable in the external circuit depends on the R_E/R_j and I_R/I_0 relations and it is therefore essential when referring to the temperature effect to state these quantities It seems that the temperature coefficient can take two values, a real and an apparent one

The *real temperature coefficient of the radiation generated current* can be measured in a circuit configuration in which $R_F/R_j \ll 1$ and with irradiation such that $I_R/I_0 \gg 1$ In any other situation the observed temperature response is an apparent one and it may differ considerably from the real one not only in magnitude but in direction as well

In clinical measurements in which the detector is faced with variable ambient temperature a compensated external circuit configuration should be used Only detectors with positive response to temperature in the exposure range of interest qualify for this application The compensation is achieved by setting the operating point at the cross-over point of the temperature characteristic ($R_E = R_c$) However, the presence of the thermal transient wave must be taken into consideration

Acknowledgements

The author wishes to thank Dr M Cohen for helpful advice stimulating comment during the course of the work and reading the manuscript A number of detectors used in these investigations were loaned by A E R E Harwell (Mr I L Allsworth) and I wish to record my gratitude for this assistance

SUMMARY

The response to temperature of silicon p n junction surface barrier devices used as dosemeters in dc short circuit mode of operation was investigated and an analysis of their performance in a temperature compensated circuit made It was found that detectors of both planar and cylindrical geometry have similar temperature characteristic and it was shown that there are three contributors to the temperature effect the variations in the radiation generated current and changes in the thermal current and the internal impedance of the p n junction Their behaviour and contribution were studied The p n junction detectors can have both positive and negative temperature coefficient an effect not very clearly explicable by the theory of the p n junction The detectors showing an increase of current with increasing temperature qualify for operation in the compensated external circuit and are therefore useful for application in clinical measurements

ZUSAMMENFASSUNG

Es wurde die Temperaturabhängigkeit von Silikon PN Übergangs Oberflachensperrschicht detektoren die als Dosimeter in einer Gleichstrom Kurzschluss Schaltungs Arbeitweise verwendet wurde, untersucht und eine Analyse deren Funktion in einer Temperatur

kompensierten Schaltung vorgenommen Es wurde gefunden, dass Detektoren von sowohl planer als auch zylindrischer Geometrie ahnliche Temperatureigenschaften haben und es wurde gezeigt, dass drei Faktoren zum Temperatureffekt beitragen die Veranderungen in dem durch die Strahlung hervorgerufenen Strom und dem Warmestrom und der internen Impedance des PN Übergangs Deren Verhalten und Beitrag wurden untersucht Die PN-Übergangsdetektoren konnen sowohl positive als auch negative Temperatur Koeffizienten haben ein Effekt, der sich nicht vollstandig durch die klare Theorie des PN Übergangs erklaren lasst Die Detektoren, die einen Anstieg des Stroms mit steigender Temperatur zeigen sind fur die Arbeit in der kompensierten ausseren Schaltung gunstig und deshalb fur die Verwendung bei klinischen Messungen anwendbar

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FROM THE DIVISION OF RADIOBIOLOGY (DIRECTOR DOCENT A NILSSON), THE RESEARCH INSTITUTE OF NATIONAL DEFENCE, 172 04 SUNDBYBERG, SWEDEN

RADIOSTRONTIUM INDUCED EARLY CHANGES IN THE HAEMATOPOIETIC TISSUES

With special reference to the thymus in mice

BERTIL JARPLID

The investigation of radiostrontium induced changes in the haematopoietic tissue has for natural reasons been concentrated to the organs chiefly affected by this nuclide namely the bone marrow and spleen The bone marrow is exposed to a concentrated prolonged irradiation from strontium accumulated in the bone tissue This may lead, inter alia, to the development of bone marrow lymphoma (NILSSON 1971) The spleen is affected, in the same way as other organs through the initial circulation of ^{90}Sr, but will thereafter be exposed also to the demand to compensate the damaged bone marrow by means of extramedullary haematopoiesis (NILSSON 1962) Lymphoma may also develop in the thymus, and preliminary investigations indicate that the incidence of such lymphomas may increase if fractionated external irradiation is combined with injection of radiostrontium (JARPLID, to be published) Whereas the acute thymic injuries after external irradiation are fairly well known, this is not the case with the acute effects of ^{90}Sr on the thymus In the present investigation, therefore, the more short term effects of ^{90}Sr have been examined in the haematopoietic tissue paying special attention to the thymus

Submitted for publication 24 March 1972

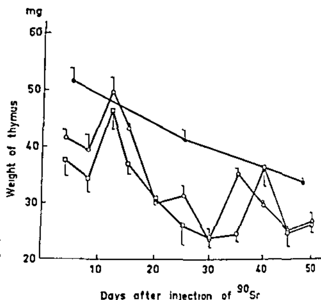


Fig 1 Weight of thymus after injection of ^{90}Sr Untreated controls (●), 0 4 μCi (○) and 0 8 μCi (□) ^{90}Sr/g body weight n for each sample = 10 Mean ± SE

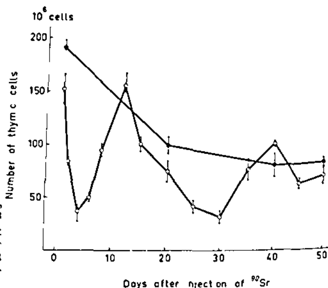


Fig 2 Total number of thymic cells within the size range 60 to 500 μ^3 after injection of ^{90}Sr The cell count was made with an electronic Coulter counter Untreated controls (●) 0 8 μCi ^{90}Sr/g body weight (○) n for each sample = 5 Mean ± SE

Material and Methods

Female CBA mice, aged 30 ± 3 days, were used The effect of different doses of ^{90}Sr was examined histologically in 220 mice, which were divided into two groups and injected with 0 4 and 0 8 μCi ^{90}Sr/g body weight, respectively From each group 10 animals were selected at random and killed at intervals of 4, 8, 12, 15, 20, 25, 30, 35, 40, 45 and 50 days after the treatment Thirty untreated

animals served as controls and were killed in groups of 10 at 5, 25 and 45 days after the start of the experiment In all animals the thymus and spleen were excised, weighed and fixed together with the sternum in Stieve s fluid for histologic examination Conventional histologic methods were used and the sections were regularly stained with Ehrlich s haematoxylin and eosin In another experiment 70 mice were injected with 0 8 μCi ^{90}Sr/g body weight and sacrificed in groups of five after 1, 2, 4, 6, 8, 12, 15, 20, 25, 30, 35, 40, 45 and 50 days Twenty mice served as untreated controls and were killed in groups of five at 1, 20 40 and 50 days after the start of the experiment At autopsy the thymus was excised and weighed and thereafter suspended in homogenisation tubes containing measured volumes of balanced salt solution (BSS) The total number of cells within the size range 60 to 500 μ^3 was calculated on an electronic counter (Coulter Counter Model B, Coulter Electronics Inc, Hialeak, Florida, USA) and the frequency distribution of the cell volumes was determined with an electronic particle size analyser (Coulter Plotter, Model J) Details of the procedure have been described earlier (JARPLID 1968) The original records from the Coulter plotter were recalculated by a Focal program in a Digital computer (Digital Equipment Corporation, Maynard Massachusetts USA) to get the mean value and standard error for cell volumes within each channel The day for injection is called day 0

Results

Thymus The changes in mean total weight of the thymus were dose dependent and revealed a diphasic process in the two highest dose groups (Fig 1) The thymic weight was not altered by 0 2 μCi ^{90}Sr until after 45 and 50 days, when it was somewhat lower than in the controls

In the 0 8 μCi group the diphasic course of the thymic weight was also reflected in the total number of thymic cells (Fig 2), the number of small lymphocytes (100 to 140 μ^3) and the histologic appearance of the thymus Both the weight and the total cell count of the thymus had their minima on days 4 and 30 and their maxima on days 12 and 40 After the last peak on day 40 the values were somewhat lower than normal on days 45 and 50 Histologically the first loss of cells resulted in a slightly narrower and less dense cortex than normal (cortical thinning, Figs 3 to 5) on day 4, corresponding to the first phase of lymphocyte depletion after roentgen irradiation (JARPLID 1968) During the first regeneration phase on days 8 and 12 the thymus had a normal histologic appearance As a result of a second depletion of cells the cortex thereafter again became narrower and less dense This proceeded until day 30 when a new regeneration set in This second phase of regeneration brought a renewed



Fig 3

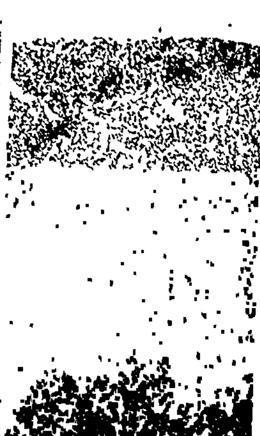


Fig 4

Fig 5

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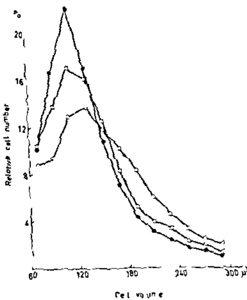


Fig 6 Comparison of cell distribution in thymic lobes Controls (●) Four days (O) and 30 days (□) after injection of 0 8 μCi ⁹⁰Sr/g body weight n for each sample = 5

increase in cell count and total weight of the thymus up to day 40 by which time the histologic appearance in general had also become normal The histologic changes were bilateral and symmetrical within the thymus.

In the 0 4 μCi group the changes in weight of the thymus were reflected histologically only slightly in a few animals No histologic changes were observed in the thymus after treatment with 0 2 μCi ^{90}Sr

Cell volume distributions of single thymus from each time after treatment had, as did the control thymus, the same main course and were recalculated together The majority of the thymic cells were of the order of 100 to 140 μ^3, manifestly corresponding to the small lymphocytes (Fig 6) After injection of ^{90}Sr there were no remarkable changes in the volume distribution on day 1 or 2 in spite of the fact that the total number of thymic lymphocytes on day 2 had decreased to about half the normal value On the 4th day the peak for small lymphocytes was depressed at the same time as the proportion of larger cells was increased Two days later the volume distribution was normal again and remained so during the period up to day 20 Thereafter the peak for small


Fig 3 Thymus I nterest ...
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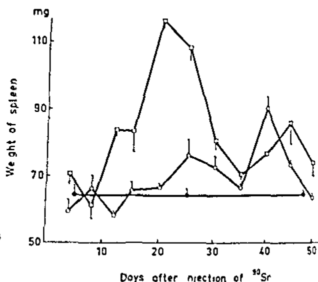


Fig 7 Weight of spleen after injection of 90Sr Untreated controls (●) 0 4 μCi (○) and 0 8 μCi (□) 90Sr/g body weight n for each sample = 10 Mean ± SE

lymphocytes was moderately lowered on days 25 and 30 and on the 50th day

Spleen The changes in weight of the spleen were also dose-dependent Thus the spleen weight was influenced by injection of 0 4 and 0 8 μCi/g body weight (Fig 7) but not by injection of 0 2 μCi A moderate increase in weight was noted on days 40 to 45 in the 0 4 and 0 8 μCi groups and a heavy increase was seen on days 25 and 30 in the 0 8 μCi group Histologically the red pulp in both these groups was characterized by a more intense extramedullary haematopoiesis than normal during the whole of the observation period (Figs 8, 9) This haematopoiesis was, however, greater in the 0 8 μCi than in the 0 4 μCi group, with a maximum on days 20 to 30 In the 0 2 μCi group the extramedullary haematopoiesis was moderately increased during the period 4 to 12 days after treatment Initially the extramedullary haematopoiesis was dominated by erythrocytic precursors In the 0 8 μCi group there was a moderate depletion of lymphocytes in the lymphoid follicles during the period 4 to 30 days (Figs 8, 9)

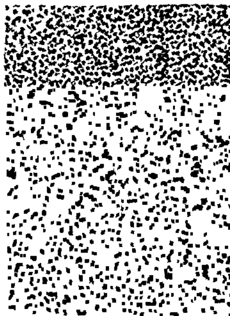
Sternal bone marrow The acute radiation injury was characterized by dilatation of the medullary sinusoids, haemorrhages, and reduced number of haem

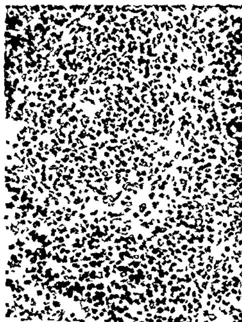
Fig 8 Spleen Untreated control mouse 25 days after start of experiment Normal cellularity of lymphoid follicle above Red pulp below H & E × 300

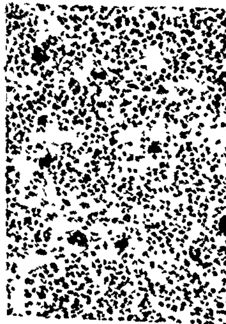
Fig 9 Spleen Twenty five days after injection of 0 8 μCi 90Sr/g body weight Follicle with lymphocyte depletion above Red pulp with increased extramedullary haematopoiesis below H & E × 300

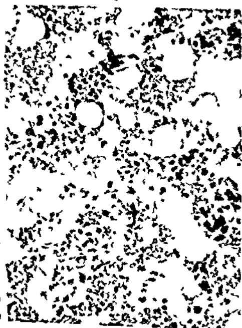
Fig 10 Bone marrow Untreated control mouse five days after start of experiment H & E × 300

Fig 11 Bone marrow Twelve days after injection of 0 8 μCi 90Sr/g body weight Dilated sinusoids haemorrhages and depletion of haematopoietic cells H & E × 300


Fig 8


Fig 9


Fig 10


Fig 11

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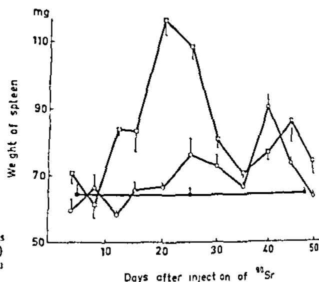


Fig 7 Weight of spleen after injection of ^{90}Sr Untreated controls (●) 0.4 μCi (○) and 0.8 μCi (□) ^{90}Sr/g body weight n for each sample = 10 Mean ± SE

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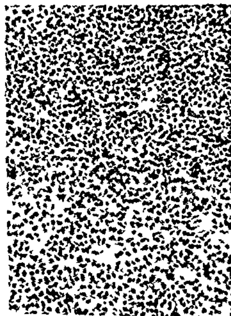
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Fig 8

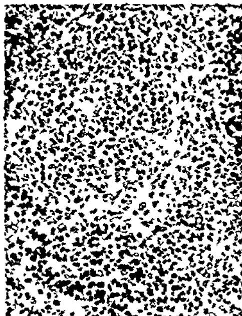
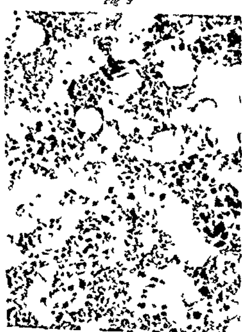

Fig 9


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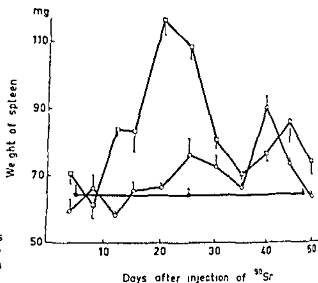


Fig 7 Weight of spleen after injection of ^{90}Sr Untreated controls (●) 0 4 μCi (○) and 0 8 μCi (□) ^{90}Sr/g body weight n for each sample = 10 Mean ± SE

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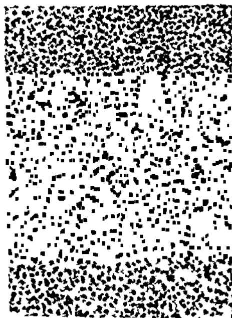


Fig 8

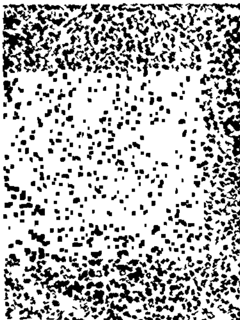


Fig 9

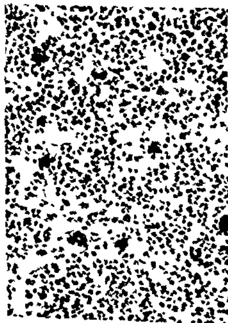


Fig 10

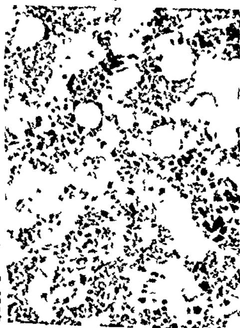


Fig 11

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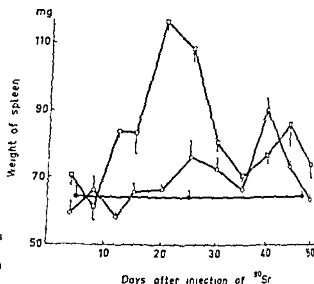


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Fig 11 Bone marrow Twelve days after injection of 0 8 μCi ^{90}Sr/g body weight Dilated sinusoids, haemorrhages, and depletion of haematopoietic cells H & E × 300

& BROWN 1952, BROWN et coll 1953, KAPLAN et coll 1953, JARPLID 1968) It is not entirely clear how the protected bone marrow affects the thymus in this respect, even if certain data indicate that it supplies the thymus with stem cells (POPP 1961, FORD & MICKLEM 1963, METCALF 1966, WALLIS et coll 1966) If this is so, the delayed regeneration of the thymus might in such case be caused by the strontium induced damage to the bone marrow It is also possible that certain parts of the thymus may be affected by radiation from strontium accumulated in surrounding bone

The changes of the spleen and bone marrow were similar to those described by NILSSON (1962) The sternal bone marrow investigated here, however, was probably exposed to radiation of a relatively lesser degree than those parts of the bone marrow which were investigated by NILSSON

The splenic hyperplasia was dose dependent, as was also noted by NILSSON (1970) after treatment with ^{90}Sr and by BRUES & STROUD (1964) after external irradiation The hyperplasia was a result of compensatory extramedullary haematopoiesis, which probably reflects the degree of bone marrow destruction The lymphocyte depletion of the spleen follicles was probably an indirect effect of the strontium via the bone marrow or the thymus

Acknowledgement

This investigation was carried out as part of the programme of the European Late Effects Project Group (EULEP)

SUMMARY

The effects of radiostrontium on the haematopoietic tissues have been examined 1 to 50 days after treatment Special interest was devoted to the thymus, which after treatment regenerated in two phases

ZUSAMMENFASSUNG

Die Wirkungen von Radiostrontium auf die hamatopoietischen Gewebe wurden 1 bis 50 Tage nach der Behandlung untersucht Besonderes Interesse wurde dem Thymus gewidmet, der nach der Behandlung in zwei Phasen regenerierte

RÉSUMÉ

Les effets du radiostrontium sur les tissus hematopoietiques ont ete examines de 1 a 50 jours apres le traitement Le thymus a été particulierement etudie apres le traitement il régenere en deux phases

atopoietic cells (Figs 10, 11) The degree of these changes varied between different parts of the sternal marrow In the 0 8 μCi group these changes were of a moderate degree by days 4 and 8 On the 12th day the sinusoids were destroyed, with extensive haemorrhages Some focal regeneration was also seen During the period 15 to 20 days the haemorrhages began to disappear, at the same time as the marrow became more fatty and the regeneration increased On days 35 to 39 the marrow was again fairly normal histologically

After treatment with 0 2 and 0 4 μCi ^{90}Sr the cellular depletion, the dilatation of the sinusoids and the haemorrhages were of slight degree during the period 4 to 12 days A successive normalization thereafter led to a relatively normal histology of the sternal marrow by days 20 and 20 to 29 respectively

Discussion

Injection of strontium quickly led to a reduced cellularity, with reduced size and weight of the thymus, which thereafter regenerated in two phases (Figs 1, 2) The conditions broadly resemble those seen in connection with external whole-body irradiation (JARPLID 1968) Two days after strontium treatment the volume distribution of the thymic cells was still normal although both the number of the smaller lymphocytes (100 to 140 μ^3) and the total number of lymphocytes had decreased to about half of the normal (Fig 2) This indicates that the reduction of the cell count applied to all sizes of cells This contrasts with the effect of external irradiation, after which the reduction of the cell count after 2 days related chiefly to the small lymphocytes (JARPLID 1968) This difference in effect may possibly indicate that the initial action of strontium on the thymus is chiefly indirect via, for example, the bone marrow But further investigations would be required to bring clarity in this matter

On day 4 it was chiefly cells less than 150 μ^3 that had been reduced in number, while the proportion of larger cells had increased, probably due to an incipient regeneration (Fig 6) Only two days later the volume distribution of the thymic cells was again normal despite an insignificant increase in cell count and in weight of the thymus The probable explanation of this is that larger cells had been differentiated into smaller

The second depletion phase after day 12 continued until day 30, i e about 1 week longer than after external irradiation (JARPLID 1968) The histologic picture and the volume distribution of the cells during that period, however, were largely similar after both methods of irradiation Earlier experiments have shown that the protection of active bone marrow during irradiation or the injection of viable bone marrow cells after irradiation promotes the regeneration of the thymus and prevents the occurrence of a second depletion phase (KAPLAN

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Table 1

Colony formation and ^{59}Fe uptake in spleens and femora of recipient mice after injection of 1×10^5 pooled bone marrow cells from 5 splenectomized donors in each group

Weeks after ^{226}Ra injection	Group of tested mice	No of recipient mice	No of colonies (mean ± SE)	Per cent 18 hour ^{59}Fe uptake into recipient spleens (mean ± SE)	Per cent 18 hour ^{59}Fe uptake into 2 recipient femora (mean ± SE)
5	^{226}Ra	—	—	—	—
	Controls	12	18.2±2.5	2.65±0.38	0.38±0.03
9	^{226}Ra	12	11.5±2.3	1.20±0.19	0.44±0.04
	Controls	—	—	—	—
14	^{226}Ra	15	9.2±0.8**	0.87±0.07	0.33±0.03
	Controls	16	21.4±1.9	1.86±0.26	0.39±0.04
18	^{226}Ra	20	12.7±1.5	1.27±0.18*	0.29±0.03
	Controls	16	20.0±2.8	2.38±0.39	0.38±0.03
40	^{226}Ra	19	8.5±0.9**	0.85±0.09**	0.33±0.01
	Controls	16	16.4±1.3	1.50±0.13	0.37±0.05

* Significantly different from control value at 95 % level

** Significantly different at 99 % level (t test)

Table 2

Peripheral blood counts femoral marrow cellularity and femoral colony forming units in splenectomized mice (5 animals in each group)

Weeks after ^{226}Ra injection	Group of tested mice	Erythrocytes/ mm³ (mean ± SE) × 10⁶	Leukocytes/ mm³ (mean ± SE) × 10³	Nucleated marrow cells per femur × 10⁶	Total units per femur × 10³
5	^{226}Ra	9.7±0.4	16.3±2.4	26.4	—
	Controls	9.9±0.5	9.2±0.6	24.0	4.4
9	^{226}Ra	9.7±0.2	9.9±0.7	19.4	2.2
	Controls	9.7±0.5	12.5±0.8	26.0	—
14	^{226}Ra	9.2±0.3	9.7±0.9	22.6	2.1
	Controls	9.2±0.3	10.6±1.3	21.4	4.6
18	^{226}Ra	8.5±0.2	10.0±1.0	22.6	2.9
	Controls	8.5±0.1	14.8±1.4	31.6	6.3
40	^{226}Ra	9.3±0.5	9.6±0.5	26.2	2.2
	Controls	9.5±0.2	8.0±1.2	37.8	6.2

before the intraperitoneal injection of ^{226}Ra, the radiation from which failed to influence the survival of animals in the course of the experiment The results obtained could be compared with the data of the previous investigation in nonsplenectomized mice, contributing thus to the evaluation of the role of the spleen in the hemopoiesis of intact and irradiated mice

Material and Methods The experiment was carried out in female random bred H-mice weighing approximately 25 g and housed in groups of five under specific pathogenic-free conditions Splenectomy was performed in mice 8 weeks old under hexobarbital anesthesia (0 15 mg/g body weight) Two weeks later, one group of these mice was injected intraperitoneally with 0 03 μCi ^{226}Ra/g body weight, another group being maintained as control

Peripheral blood counts and blood smears were performed at intervals of 5, 9, 14, 18 and 40 weeks after the injection, and the whole-body activity was measured by the method of LENGER (1971), five mice were examined at each interval The animals were killed by cervical transection and the right femora dissected The suspension of marrow cells was prepared by grinding the femora in a mortar, and washing the cells out of the bones with 10 ml Tyrode solution The cellularity of the pooled femoral marrow was then determined

Recipients of the same sex, weight and origin and all 10 weeks of age were exposed to 800 R whole-body irradiation at 60 R/min, HVL 1 0 mm Cu and a target distance of 50 cm with a TUR roentgen unit The animals were placed in the homogenized field of a rotating plastic container and the system was monitored with an Integron-Victoreen ionization chamber Immediately after exposure the lateral tail veins were injected with 1×10^5 marrow cells of the tested mice One group received the cells from ^{226}Ra treated mice and another group those from non irradiated controls

The recipient mice were killed on the ninth day, 18 hours before which they were injected intraperitoneally with 1 μCi of ^{59}Fe (as ferric citrate) The spleens as well as both femora were extirpated, fixed in Bouin s solution, and counted in a well-type gamma detector The uptake of ^{59}Fe in the spleens and femora was expressed as a percentage of the activity administered Spleen colonies were counted independently by three observers 24 hours later The total colony forming units per femur of the donor mice were calculated on the basis of the relative colony forming units and the total nucleated cell content in the femur

The experiment was supplemented with histology of the liver and of certain other tissues carried out in 7 splenectomized mice one year later, 3 of these having received ^{226}Ra and 4 control animals The liver of these mice was also examined cytologically

Table 1

Colony formation and ^{59}Fe uptake in spleens and femora of recipient mice after injection of 1×10^5 pooled bone marrow cells from 5 splenectomized donors in each group

Weeks after ^{226}Ra injection	Group of tested mice	No of recipient mice	No of colonies (mean ± SE)	Per cent 18 hour ^{59}Fe uptake into recipient spleens (mean ± SE)	Per cent 18 hour ^{59}Fe uptake into 2 recipient femora (mean ± SE)
5	^{226}Ra	—	—	—	—
	Controls	12	18.2 ± 2.5	2.65 ± 0.38	0.38 ± 0.03
9	^{226}Ra	12	11.5 ± 2.3	1.20 ± 0.19	0.44 ± 0.04
	Controls	—	—	—	—
14	^{226}Ra	15	9.2 ± 0.8**	0.87 ± 0.07	0.33 ± 0.03
	Controls	16	21.4 ± 1.9	1.86 ± 0.26	0.39 ± 0.04
18	^{226}Ra	20	12.7 ± 1.5	1.27 ± 0.18*	0.29 ± 0.03
	Controls	16	20.0 ± 2.8	2.38 ± 0.39	0.38 ± 0.03
40	^{226}Ra	19	8.5 ± 0.9**	0.85 ± 0.09**	0.33 ± 0.01
	Controls	16	16.4 ± 1.3	1.50 ± 0.13	0.37 ± 0.05

* Significantly different from control value at 95 % level

** Significantly different at 99 % level (t test)

Table 2

Peripheral blood counts femoral marrow cellularity and femoral colony forming units in splenectomized mice (5 animals in each group)

Weeks after ^{226}Ra injection	Group of tested mice	Erythrocytes/ mm³ (mean ± SE) × 10⁶	Leukocytes/ mm³ (mean ± SE) × 10³	Nucleated marrow cells per femur × 10⁶	Total units per femur × 10³
5	^{226}Ra	9.7 ± 0.4	16.3 ± 2.4	26.4	—
	Controls	9.9 ± 0.5	9.2 ± 0.6	24.0	4.4
9	^{226}Ra	9.7 ± 0.2	9.9 ± 0.7	19.4	2.2
	Controls	9.7 ± 0.5	12.5 ± 0.8	26.0	—
14	^{226}Ra	9.2 ± 0.3	9.7 ± 0.9	22.6	2.1
	Controls	9.2 ± 0.3	10.6 ± 1.3	21.4	4.6
18	^{226}Ra	8.5 ± 0.2	10.0 ± 1.0	22.6	2.9
	Controls	8.5 ± 0.1	14.8 ± 1.4	31.6	6.3
40	^{226}Ra	9.3 ± 0.5	9.6 ± 0.5	26.2	2.2
	Controls	9.5 ± 0.2	8.0 ± 1.2	37.8	6.2

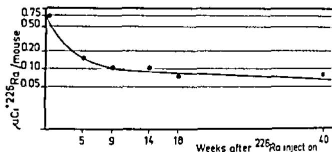


Fig 1 Retention curve for splenectomized mice after single administration of 226Ra

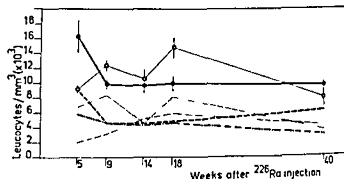


Fig 2 Course in time of white blood count in irradiated (——— total counts, — — granulocytes, — — — lymphocytes) and control splenectomized mice (——— total counts — — granulocytes, — — — lymphocytes)

Results

The results are summarized in Tables 1 and 2, while the values of the whole-body measurements taken in the course of the experiment appear in Fig 1

The counts of superficial spleen colonies were significantly lower in recipients injected with 1×10 marrow cells from splenectomized 226Ra treated mice than in those administered with cells from non-irradiated but splenectomized mice Evaluation of the splenic uptake of 59Fe in recipient mice revealed similar significant differences It is noteworthy that analogous results were not evident in the case of femoral 59Fe incorporation Under the given conditions the femoral iron uptake was almost as low as in recipient mice subjected only to roentgen irradiation

The differential blood counts per mm^3 were obtained from blood smear evaluations and total leukocyte counts When comparing the mice given 226Ra with the controls, no significant difference was evident in the granulocyte, lymphocyte and monocyte counts (Fig 2) Similarly the red blood count did not change significantly throughout the experimental period of 40 weeks Femoral cellularity of both the radium injected and control mice was maintained at between 20 and 25 million from the fifth to the fourteenth week of the experiment In the following two intervals the cellularity in 226Ra given mice remained unchanged, while that of controls exceeded the level of 30 million



Fig 3 Liver imprint with binuclear hepatocyte and intermediate erythroblast (splenectomized mouse one year after ^{226}Ra injection)



Fig 4 Early precursors of red and white series in liver imprint (splenectomized mouse one year after ^{226}Ra injection)

The number of femoral colony forming units in mice exposed to ^{226}Ra was significantly lower than in the controls during the whole experimental period The difference was manifested more clearly in the two last intervals

Histology produced evidence of hemopoiesis in the liver of three splenectomized mice killed one year after ^{226}Ra application Young granulocytes and erythroblasts were present in liver imprints stained with May Grunwald Giemsa (Figs 3, 4) In organs and tissues such as lymph nodes, thymus, kidneys, suprarenals, and lungs the hemopoiesis was absent The femoral marrow cells were seriously depressed in these three mice The early hemopoietic precursors were, however, not present in the liver of four splenectomized controls killed at the same age, only sporadic cells in more mature stage being evident In no other organ or tissue was ectopic hemopoisis observed

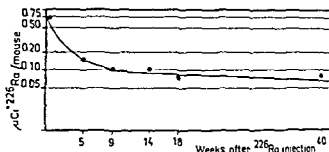


Fig 1 Retention curve for splenectomized mice after single administration of ²²⁶Ra

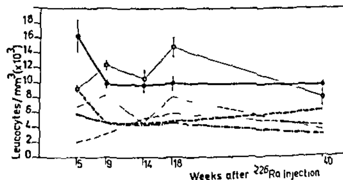


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values decreased to 0.70. No significant changes occurred in the red and white peripheral blood counts during the experiment. This means that the normal level of the peripheral blood count can be maintained by hemopoiesis continuously damaged by the internally deposited ^{226}Ra and weakened by the elimination of spleen blood forming function. This observation seems to be in agreement with the findings of LISI et coll. (1969) in BALB/c female mice, indicating that the normal spleen has little influence on overall murine erythropoiesis.

It is however recognized that latent splenic factors exist in mouse hemopoiesis and become evident under stress conditions of acute and subacute irradiation. Classic experiments by JACOBSON et coll. (1949, 1950) indicated that the compensatory blood formation appeared in the spleen shielded during whole-body exposure to 600 or 1025 R roentgen irradiation. FRIED et coll. (1966) found that damage to bone marrow in ^{89}Sr applied mice was followed by the proliferation of spleen hemopoietic stem cells.

Noncellular factors producing postirradiation recovery of hemopoiesis were isolated both from the nonirradiated and irradiated spleen. Experiments by FORD et coll. (1968) in mice revealed a cell free splenic extract effective upon the postirradiation survival. KNOSPE et coll. (1970) investigated a noncellular substance prepared from irradiated spleen and inducing stem cell proliferation in CAF_1 and CF_1 mice exposed to roentgen irradiation. Certain investigators (BURGER et coll. 1969, ŠMID & ŠIMŠA 1971) identified alpha-2 globulin of splenic origin as being capable of stimulating the hemopoietic recovery after irradiation. According to TWENTYMAN & BLACKETT (1970) spleen might be responsible for additional erythropoiesis and simultaneously for the maintenance of normal marrow erythropoiesis. The absence of the spleen may lead to changes in iron kinetics. Thus, both cellular and noncellular protective mechanisms were demonstrated in the spleen without excluding one another.

The present long term experiments also revealed that a certain positive relationship between spleen and bone marrow hemopoiesis existed under normal conditions. If the relation is interrupted by splenectomy the number of both colony forming units and nucleated bone marrow cells is decreased and a new equilibrium state is supposed to be established. The same is also evident in mice the bone marrow of which is being continuously exposed to radiation from incorporated ^{226}Ra. The splenic mechanisms influencing favourably the bone marrow hemopoiesis seem to be radiation resistant as in other homeostatic systems.

During the experimental period the absence of the spleen in ^{226}Ra injected mice did not interfere with the compensatory mechanisms of marrow hemopoiesis to such an extent that the production of blood cells became insufficient. The



Fig 5 Colony forming units in femoral bone marrow of ^{226}Ra injected splenectomized (black bar), ^{226}Ra injected nonsplenectomized (shaded bar), and nonirradiated splenectomized mice (heavy line bar) as compared with respective values for intact controls (100 %) (light line bar)

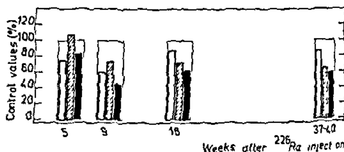


Fig 6 Cellularity of femoral bone marrow of ^{226}Ra injected splenectomiezd (black bar), ^{226}Ra injected nonsplenectomized (shaded bar), and nonirradiated splenectomized mice (heavy line bar) as compared with respective values for intact controls (100 %) (light line bar)

Discussion

The present investigation in splenectomized mice given ^{226}Ra was carried out under the same conditions as in the previous experiment in the similarly treated nonsplenectomized animals (SVOBODA & KLENER) It proved useful to summarize the data from both experiments and to compare the corresponding groups (Figs 5, 6) The number of femoral colony forming units in the splenectomized, as compared with nonsplenectomized controls, fell during the whole experimental period and amounted in the last two intervals to 0 71 and 0 75 of their reference values A comparison of the splenectomized and nonsplenectomized mice given ^{226}Ra produced analogous differences, these being most marked in the last interval (factor 0 5) The evaluation of femoral marrow cellularity revealed similar changes although of a lesser degree than with the colony forming units. Peripheral blood counts remained at the normal level in all groups examined

The experiment indicated that the femoral compartment of pluripotent stem cells and of early differentiated progenitors capable of colony formation was impaired more seriously in mice with eliminated splenic hemopoiesis and exposed to ^{226}Ra Nevertheless, this compartment did not fail and amounted to 0 36 to 0 50 of the control values in splenectomized but non irradiated animals Such a depression was not observed in the femoral cellularity of splenectomized and radium injected mice in which only at the last two intervals the respective

ZUSAMMENFASSUNG

Es wird über hämatologische Daten von splenektomierten Mäusen während langer Zeiträume nach intraperitonealer Injektion von ^{226}Ra (0,03 μCi/g) berichtet. Die Kolonie bildenden Einheiten des Knochenmarks waren während der gesamten Versuchszeit herabgesetzt. Die peripheren Blutwerte waren nicht signifikant verändert.

RÉSUMÉ

Présentation des données hématologiques obtenues sur des souris splénectomisées au cours de longues périodes suivant l'injection intrapéritonéale de ^{226}Ra (0,03 μCi/g). Les unités formatrices de colonie dans la moelle osseuse sont en nombre réduit tout au long de la période expérimentale. Les numérations du sang périphérique ne sont pas significativement modifiées.

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ectopic hemopoiesis was observed one year after radium injection, which fact could be explained by the increasing inability of the bone marrow to produce the required amount of peripheral blood elements.

An intact spleen may form the centre for the compensatory blood forming activity. Preliminary results of the experiment with higher ^{226}Ra activities suggest that with heavily damaged marrow the spleen hemopoiesis is activated although unable to prevent the failure of overall hemopoiesis.

The results are worth comparing with the findings in C3H mice of KRETCHMAR & CONOVER (1970) who reported seven times higher concentration of colony forming units in the bone marrow than in the spleen. They assumed in addition that the ratio of colony forming units having pluripotent properties to all other such units including 'early differentiated precursors' is three times higher in the bone marrow than in the spleen. RENCRICCA et coll. (1970) demonstrated in mice with phenylhydrazine induced anemia that the units migrate from the bone marrow into the spleen rather than proliferate locally. These facts indicate a restricted ability of the spleen of the mouse to compensate for the damaged function of the bone marrow, and thus illustrate some important differences in the hemopoietic capacity between the spleen and bone marrow.

An additive hemopoietic function of the spleen in relation to the bone marrow is presumed as being effective both in non-irradiated and radium injected mice. The participation of such a splenic factor remains unchanged as long as the bone marrow maintains the production of differentiated elements. In the case of marrow decompensation signs of increased spleen hemopoiesis proportional to the marrow damage may occur. If the spleen be removed the ectopic blood formation in other tissues is established as the only available but not fully effective compensatory mechanism. The spleen is an important organ for hemopoiesis in mouse under both normal and stress conditions. The parts played by the complementary cellular proliferation and the homeostatic mechanisms remain, however, to be elucidated in greater detail.

Acknowledgements

The authors wish to thank Mrs R. Housová and Mrs Z. Kotašková for their technical assistance.

SUMMARY

Hematologic data obtained in splenectomized mice for long periods after the intraperitoneal injection of ^{226}Ra (0.03 μCi/g) are reported. The colony forming units in the bone marrow were reduced throughout the whole experimental period. The peripheral blood counts were not changed significantly.

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CARCINOMA OF THE VULVA

Results of an individualized treatment schedule

B FRANKENDAL, L G LARSSON and P WESTLING

Carcinoma of the vulva is a relatively rare malignant condition and constitutes about four per cent of all gynaecologic carcinomas and one per cent of all new malignant growths in women (Cancer Registry of Sweden 1971) As the vulva and the lymph nodes of the groin are accessible for early diagnosis and radical treatment good prognosis might be expected in this disease However, carcinoma of the vulva mostly affects women over sixty years of age, less prone to be so worried by the symptoms such as pruritus or ulceration and the condition is therefore often in a relatively advanced stage when the patient comes under treatment

Radical vulvectomy, including bilateral dissection of the inguinal lymph nodes, has many advocates The mortality of the operation in an unselected material has been reported however to be as high as 19 per cent (WAY 1960) Various modifications of the operation have been proposed in order to diminish this rate and improve the results Individualized treatment in which the extension of the operation is determined by the clinical stage of the growth and the physical state of the patient seems to give good results (RUTLEDGE et coll 1970) The wide spread precancerous lesions often present in the vulva together with the latent

Submitted for publication 13 September 1972

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condition usually suggest, however, total vulvectomy in most cases (LUNDWALL 1961)

Electrocoagulation of the vulva was introduced about 1922 (BERVEN 1941) and is still used in some centres It is as radical a procedure as total vulvectomy and may be applied in patients in poor physical condition Inguinal lymph node dissection may be combined with electrocoagulation of the primary growth but is then usually postponed until the coagulation wound has healed

Radiation treatment of the primary growth has been described by several authors (BERVEN 1941, JOHNSSON 1964, ALMFNDRAL 1972) General experience has been that the vulvar tissues are relatively sensitive to irradiation, resulting in a fairly large number of complications, mainly necrosis No doubt exists that high energy photons or electrons from supervoltage machines have increased the possibilities of adequate radiation treatment of carcinoma of the vulva Irradiation is especially valuable in advanced neoplasms in which total vulvectomy or electrocoagulation may be impossible or attended by too great risks of complications Some authors (SCHUBERT et coll 1960) have even used radiation treatment in early operable cases, but surgical methods in this group are generally preferred

The treatment of the regional lymph nodes presents a complicated problem The frequency of lymph node metastases varies in different materials from 45 to 57 per cent (TAUSSIG 1940, WAY 1951, EDSMYR 1962, BOUTSELIS 1972) Clinically manifest lymph node metastases may be treated by surgical dissection irradiation or a combination of both In cases without clinically established lymph node metastases, some authors use so-called prophylactic bilateral dissection of the lymph nodes of the groin (inguinal nodes, femoral nodes and sometimes iliac nodes) or radiation treatment of the inguinal regions for eradication of sub-clinical metastases As the frequency of lymph node involvement is largely dependent upon the size and site of the primary tumour (EDSMYR 1962, BOUTSELIS 1972) and on the degree of tumour differentiation (WAY 1951), some centres have a more individualized approach For example, when the primary tumour is situated laterally in the vulva, the frequency of metastases in the contralateral nodes is much lower than in the homolateral nodes Radiumhemmet, Stockholm which has largely influenced the treatment of carcinoma of the vulva, has employed the following method (BERVEN 1941) After electrocoagulation of the vulva, both groins receive radiation treatment, followed by lymph node dissection in which clinically manifest metastases are present, so-called prophylactic dissection is not performed A detailed report of 560 cases treated in this way was published by EDSMYR (1962)

An individualized treatment schedule has been used since 1959 in our department, the stage of the condition and the physical state of the patient

Table 1

Treatment method and clinical staging in patients with squamous cell carcinoma of the vulva

Primary tumour	Inguinal lymph nodes	No of cases	Stage I	II	III	IV	Lymph node metastases (microscopically verified) None	Unilat	Bilat
Electrocoagulation		16	9	5	2	—	13	3	—
	+homolateral inguinal radiation treatment	12	—	6	3	3	6	6	—
	+bilateral inguinal radiation treatment	11	—	1	10	—	8	2	1
Radical excision		5	3	2	—	—	5	—	—
	+homolateral inguinal radiation treatment	1	—	—	—	1	—	5	—
	+bilateral inguinal radiation treatment	—	—	—	—	—	—	—	—
Radiation		4	3	—	—	1	4	—	—
	+homolateral inguinal radiation treatment	2	—	—	1	1	1	1	—
	+bilateral inguinal radiation treatment	4	—	—	1	3	1	—	3
No treatment		2	—	—	—	2	—	—	2
Total		57	15	14	17	11	38	13	6

having determined the type The purpose of the present investigation was to assess the results of this schedule of treatment

Material The Department of Radiation therapy serves a population of about 650 000 During the period 1959 to 1971 a total of 73 patients with malignancy of the vulva were admitted The department was consulted regarding 3 additional patients who were not admitted (Two of these had basal cell carcinomas and one had a malignant melanoma) Eight of the patients admitted had been treated elsewhere for the primary growth and had a recurrence Of the 65 primary patients 57 had squamous cell carcinomas, 4 had malignant melanomas, 2 had basal cell carcinomas, one had morbus Bowen, and one a malignant apocrine neoplasm

This report deals mainly with 57 patients with squamous cell carcinomas, 55 of whom were treated The mean age of the group was 64 years, the youngest patient was 28 and the oldest 91 years old Twenty-two of these patients (38 6 per cent had palpable inguinal nodes probably containing metastases from the

Table 2

Time elapsed from the onset of the symptoms until admission Data from 54 patients

Duration of history, years	No of patients
> 0 – 0.5	20
> 0.5 – 1	19
> 1 – 1.5	3
> 1.5 – 2	4
> 2 – 2.5	1
> 2.5 – 3	3
> 3 – 3.5	–
> 3.5 – 4	1
> 4 – 4.5	–
> 4.5 – 5	1
> 5	2

vulva The presence of metastases was verified in 19 of these patients, either by cytology after fine needle biopsy or by histology after removal In 35 patients no inguinal nodes could be palpated or they were classified as normal (Table 1)

Length of history Only signs and symptoms that could possibly be connected with the condition were taken into account Fifty-four patients could give an adequate history Pruritus was the most frequent symptom and was complained of by 18 of 54 patients, some however had had pruritus for many years and had been examined on repeated occasions with negative results before diagnosis of the tumour, pruritus was therefore not included in determining the duration of the history (Table 2)

Other malignant conditions Five of the 57 patients with epidermoid carcinoma had been — or were later on — treated for another malignant condition, 2 had cervical carcinoma, one carcinoma of the uterine body and one carcinoma of the breast Another patient was treated for a growth of the buccal mucosa some years after treatment of her vulvar carcinoma Further, one patient who was treated for morbus Bowen of the vulva had earlier been treated for carcinoma of the cervix uteri

Classification A retrospective classification of the patients with squamous cell carcinoma has been according to the TNM system proposed by UICC 1967,

Table 3

Complications of the treatment that required intervention

Treatment	Total No of patients treated	Type of complication	No of patients
Electrocoagulation	39	Adhesion of introitus vaginae	2
		Postoperative hemorrhage	3
		Edema of the vulva	1
		Vulvar infection	1
Irradiation	10	Severe radiation reaction	3
Radical excision	6		0
Total	55		10

from which EDSMYR & KOTTMEIER (1971) have developed a method of clinical staging The material has been grouped into 4 stages, the neoplasms were also classified microscopically as highly differentiated (40), fairly well differentiated (11) and poorly differentiated (6)

Treatment This was individualized according to the extension and type of the primary tumour and the age and state of the patient The primary tumour was usually treated by electrocoagulation of the whole vulva (BERVEN 1941) However, in younger patients and especially in those with more limited growths, wide local excision or hemivulvectomy was sometimes preferred, those cases regarded as inoperable received radiation treatment The total tumour doses varied between 6 000 and 6 500 rad over 40 to 60 days with daily treatments five days per week usually with a ^{60}Co kilocurie unit or with high energy electrons from a betatron (15 to 30 MeV)

Clinical lymph node metastases were treated mostly by dissection, often combined with pre- or postoperative radiation treatment, with no clinical metastases, prophylactic radiation treatment was withheld if the primary tumour was small and highly or fairly well differentiated Patients with medium sized or large primary tumours and all those with poorly differentiated tumours received prophylactic radiation treatment to the groins, in well lateralized tumours, however, this treatment was confined to the homolateral side The total dose of radiation administered to the inguinal regions varied between 3 000 and 6 000 rad over 15 to 55 days in daily treatments of 5 days per week

Twelve patients had unilateral (10) or bilateral (2) lymph node dissections of the groin, in 9 of these in close connection with the primary treatment, while

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Time elapsed from the onset of the symptoms until admission Data from 54 patients

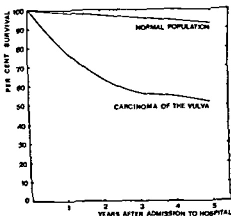
Duration of history, years	No of patients
> 0 –0 5	20
> 0 5–1	19
> 1 –1 5	3
> 1 5–2	4
> 2 –2 5	1
> 2 5–3	3
> 3 –3 5	—
> 3 5–4	1
> 4 –4 5	—
> 4 5–5	1
> 5	2

vulva The presence of metastases was verified in 19 of these patients, either by cytology after fine needle biopsy or by histology after removal In 35 patients no inguinal nodes could be palpated or they were classified as normal (Table 1)

Length of history Only signs and symptoms that could possibly be connected with the condition were taken into account Fifty-four patients could give an adequate history Pruritus was the most frequent symptom and was complained of by 18 of 54 patients, some however had had pruritus for many years and had been examined on repeated occasions with negative results before diagnosis of the tumour, pruritus was therefore not included in determining the duration of the history (Table 2)

Other malignant conditions Five of the 57 patients with epidermoid carcinoma had been — or were later on — treated for another malignant condition, 2 had cervical carcinoma, one carcinoma of the uterine body and one carcinoma of the breast Another patient was treated for a growth of the buccal mucosa some years after treatment of her vulvar carcinoma Further, one patient who was treated for morbus Bowen of the vulva had earlier been treated for carcinoma of the cervix uteri

Classification A retrospective classification of the patients with squamous cell carcinoma has been according to the TNM system proposed by UICC 1967,



Survival curves for patients with carcinoma of the vulva and for a female population of Sweden 1963—1970 of the same average age

Survival Fifty-five patients with squamous cell carcinomata received treatment of some kind, 2 patients with advanced conditions were not treated The three- and five-year results appear in Table 4 The absolute five-year survival rate and cure rate was 54 per cent As could be expected a close correlation existed between the clinical stage and the prognosis (Table 5) A survival curve for the patients from calculations with the actuarial method and a survival curve for a Swedish female population of the same average age (BERKSON & GAGE 1950) is presented in the figure Six of the 13 patients with verified inguinal node metastases on admission were alive at five years (46 per cent) Fifteen of 26 patients without demonstrable lymph node metastases on admission were alive at five years (58 per cent)

Radiation treatment of lymph node regions Twelve patients observed for more than three years without palpable inguinal nodes considered to be metastases received radiation treatment to the homolateral (5) or to both inguinal regions (7) These may be compared with 17 patients without clinical metastases, also observed for more than three years, who received no radiation treatment to the inguinal regions In the first group no inguinal metastases were registered in the subsequent course, but in the second group 3 patients developed metastases It should be noted that those patients who received inguinal irradiation were on an average in a more advanced clinical stage than the others The difference observed thus supports the opinion that adequate radiation treatment can sterilize subclinical lymph node metastases

Table 4

Three- and five year results

	Three years	Five years
No of patients referred	44	39
No of patients treated	44	39
Alive without signs of carcinoma	24 (55 %)	21 (54 %)
Alive with carcinoma	0	0
Died with carcinoma	14	11
Died of intercurrent disease, probably without carcinoma	8	7

Table 5

Survival rates in different clinical stages

Stage	3 year survival rate		5 year survival rate	
	Alive/observed	%	Alive/observed	%
I	9/11	82	8/9	89
II	8/11	73	7/10	70
III	4/14	29	4/12	33
IV	3/8	38	2/8	25
Total	24/44	55	21/39	54
Without lymph node metastases	17/29	59	15/26	58
With lymph node metastases	7/15	47	6/13	46

in the remaining 3 patients later on due to the appearance of lymph node metastases not demonstrable on admission Eight patients received preoperative radiation treatment shortly after the electrocoagulation of the primary tumour which was regarded as especially necessary as the dissection was postponed until the coagulation wound was healed Three patients operated on later and not prophylactically irradiated received postoperative radiation treatment

Complications Only complications severe enough to require treatment were included, for example a radiation reaction necessitating admission to hospital 10 complications in all in 55 patients were registered The treatment produced no deaths (Table 3)

included 9 patients with intraepithelial carcinomas who survived five years Eighty seven per cent of the patients reported by GOPLERUD & KEETTEL (1968) had epidermoid carcinomas Finally RUTLEDGE et coll (1970) excluded 17 patients who either refused treatment or appeared only at consultations Most of the patients reported had some sort of surgical treatment

The mean duration of stay in hospital of patients treated only with electrocoagulation was 23 days BYRON (1965) reported a mean of 23 8 days for 10 patients treated by conventional surgery GOPLERUD & KEETTEL (1968) on the other hand reported a mean hospital stay of 51 days for patients in whom radical vulvectomy and groin dissection were performed

WAY (1951) has convincingly demonstrated that palpation is a poor method of diagnosing inguinal metastases TAUSSIG (1940) reported that the superficial nodes were involved in 50 per cent of the cases EDSMYR (1962) stated that histologic verification was evident in only 210 of 260 patients with nodes diagnosed as containing metastases by palpation BOUTSELIS (1972) recorded 74 per cent accuracy in positive and 87 per cent in negative examinations So-called prophylactic radiation treatment where no nodes can be palpated seems well grounded Although the present material is too small to permit any definitive conclusions the mere fact that no metastases appeared in the irradiated group compared with three in the nonirradiated group suggests that the inguinal regions should be irradiated at least when the probability of subclinical lymph node metastases seems to be large

SUMMARY

Individualized treatment was given to 57 patients with squamous cell carcinoma of the vulva according to the stage of the disease and their physical states The absolute five year survival rate of 54 per cent was comparable to that reported with extensive surgery

ZUSAMMENFASSUNG

Siebenundfunfzig Patienten mit einem Schuppenzellkarzinom der Vulva wurde eine individuelle Behandlung entsprechend dem Stadium der Erkrankung und dem korperlichen Zustand der Patienten gegeben Die absolute Funfjahres Überlebensrate von 54 % war vergleichbar mit der wie sie fur extensive Chirurgie beschrieben ist

RÉSUMÉ

Cinquante-sept malades atteintes d epithelioma pavimenteux de la vulve ont subi un traitement individualise d après le stade de leur maladie et d apres leur etat physique Le taux de survie absolue à cinq ans etait de 54 pour cent et etait comparable à celui donne par une chirurgie etendue

Table 6

Squamous cell carcinoma of the vulva Results from the literature

Authors	No of cases	5 year survival rate %
WAY (1960)	79	61
WAY (1960)	96	49
EDSMYR (1962)	657	35 5
COLLINS et coll (1963)	74	54
MCKELVEY & ADCOCK (1965)	111	56 7
GOPLERUD & KEETTEL (1968)	156	36
RUTLEDGE et coll (1970)	164	55 7
BOUTSELIS (1972)	90	52 5
FRANKENDAL et coll (1972)	57	54

Discussion

The mean duration of history has been reported to be 8 to 10 months (EDSMYR 1962, GOPLERUD & KEETTEL 1968) GUSBERG & FRICK (1970) observed in some cases a history of several years, and similar observations were made in the present series Reduction of this delay of diagnosis should certainly improve the prognosis

There are different ways of treating carcinoma of the vulva Some surgeons prefer extensive surgery as standard treatment, including radical vulvectomy and bilateral groin node dissection, sometimes including deep iliac nodes, some patients are however too old or in too poor physical condition for this extensive treatment The patients who are treated surgically are thus selected which influences the mortality rate for the operation, in some reports this is about 6 per cent (MCKELVEY & ADCOCK 1965, GOPLERUD & KEETTEL 1968) Extensive surgery of this type is attended by a high incidence of complications One possible way to lower this risk is to adapt the extension of the operation to the stage of the tumour and the physical state of the patient Another possibility is to carry out the operation in stages This is in fact done with many patients treated by the method of Radiumhemmet Combining surgery with irradiation of the inguinal regions does not seem to influence the frequency of complications The results attained in this way expressed in three- and five-year survival rates and five year cure rates are as good as those reported by authors with more extensive surgical treatment The results expressed in five year survival rates presented by some authors since 1960 are collected in Table 6 The report of EDSMYR (1962) included 30 cases without confirmation by histology COLLINS et coll (1963)

Book reviews

COMPUTERS IN RADIOTHERAPY Proceedings of the Third International Conference on Computers in Radiotherapy Glasgow, 8—10 September 1970 Edited by A S Glicksman, M Cohen and J R Cunningham British Journal of Radiology 1971, Special Report Series No 5

The first meeting in this series was held at Cambridge, England, in June 1966 The discussions dealt mainly with methods of calculations for external beams and dosage around interstitial sources extended to three dimensional treatment planning and optimization The need to include radiobiologic considerations including dose fractionation and cell survival was also discussed The second meeting, held in Chicago in September 1968, was centered on problems concerning input data, such as tumour localization and record information as well as upon computer-controlled assistance in the setting up of patients for treatment These meetings had the structure of two working groups, each consisting of half of the total membership

The third meeting held recently in Glasgow, was organized in another way about twenty working groups each made up of five to ten members, concentrated on a distinct problem and presented the results of their work at the conference The main topics discussed at five plenary sessions were (A) Man machine cooperation (B) The rationale and design of treatment (C) Collection analysis and exchange of information. (D) Trends in radiation therapy and technology (E) Human beings and computers — allies or enemies?

(A) As regards the configuration of computer systems — small dedicated computers or large time sharing systems — an unequivocal answer could not be given. More emphasis could have been laid on the probably best choice the small computer as an advanced terminal to a large one The data-display system must be interactive The way of presenting 3D treatment plans was discussed

(B) This section included papers on treatmen [illegible]
in delivered radiati [illegible]
including optimizat [illegible]
position and shape of tumour, critical organs, etc) was recommended to be in the order of ± 2 mm Tissue heterogeneity was discussed too briefly, as correction for tissue heterogeneity constitutes one of the strongest arguments for computer planning

(C) The first part of this section dealt with dissemination of computer programmes and exchange of information (including patient records), nationally and internationally Four types of the cooperative use of computer programmes were discussed simple personal exchanges publication in journals or by commercial agencies, direct cooperation in a time sharing or batch mode and regional computer service In addition, the importance of maintaining a bibliography or list of programmes in the radiation therapy field was stressed The legal responsibility for use must rest on the user of the programme

New forms for exchange of clinical and biologic data must be found International agreement must be reached on the terms to be used for describing the patient, the disease, the site the histology and stage The use of existing codes must be encouraged

The type of patient data that should be recorded was also discussed and the input system to be used too much coding should be avoided At the moment, structured language was agreed upon as the best choice

(D) Discussion of growth points in radiation therapy was concerned with fast neutron therapy cell kinetics and 3D dosimetry A lively and detailed discussion followed the

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Vol 12 Fasc 3 THERAPY PHYSICS BIOLOGY 1973 June

CARCINOMA OF THE LIP

A series of 869 cases

K JØRGENSEN, O ELBRØND and A. P ANDERSEN

A total of 869 cases of carcinoma of the lip were treated during the period 1946—1966 but those cases with recurrences following primary treatment elsewhere as well as those with a doubtful histologic diagnosis were excluded from the analysis The object was first and foremost to determine the occurrence of metastases in regional lymph nodes, as such spread is low a 20-year period had to be reviewed in order to obtain a reasonably representative series. Various other data of interest, especially concerning the primary tumour, became apparent in the course of the analysis and are presented for discussion

The series cannot be said to be representative of the incidence of the condition in a given geographic area, as some cases were treated at other centres A greater tendency to centralize the treatment appears also to have occurred during recent years It is possible to trace only a slight increase in the number of newly referred cases, in other words no major change in the incidence of the disease was evident Publications from the Danish Cancer Registry (CLEMMESEN 1965) have also indicated merely a moderate increase in the annual number of new cases

Material This consisted of 869 cases, 845 (97 2 per cent) of which were males and 24 (2 8 per cent) females. The highest mean range lay in the 60 to 70 year group (Fig 1) The lesion was always an epidermoid cell carcinoma, except-

From the Radium Centre and the E N T Department, Municipal Hospital, Aarhus University 8000 Aarhus Denmark Submitted for publication 12 October 1972

presentation of papers on automation of treatment equipment and automatic treatment control

What is the ultimate aim in automation of treatment — to adhere to the original plan by all means throughout the treatment, hoping to obtain the prescribed dose or, by making continuous corrections of the plan according to daily variations, reaching a correct summing up of the dose that actually was given?

(E) This topic evoked some provocative statements and discussion on how the computer may be accepted as a helpful tool to the clinician As Alain Laugier put it 'Besides patience and/or murder, is there any other solution how a computer-minded radiotherapist can change the habits of *his older and more traditional colleagues?*"

As the participants of this conference are actively working specialists in the field, the proceedings are a really up-to-date review of the present stage of development of computers in radiation therapy. It can be recommended as a general introduction to the whole problem and as a comprehensive survey of more specialized topics

T Moller

GUIDANCE ON THE ACTION TO BE TAKEN IN THE EVENT OF INTAKE OF RADIOACTIVE MATERIAL INTO THE BODY HPA Report Series No 2 A PRACTICAL GUIDE TO ELECTRON DOSIMETRY 5—35 MeV HPA Report Series No 4 The Hospital Physicists' Association, London 1971

These small books offer practical and valuable advice in the field of radiation therapy and protection The first recommends actions to be taken at various levels of radioactive material into the body, the second is intended to help in standardizing the techniques of electron dosimetry in British radiation therapy centres and is based on equipment and methods familiar to hospital physicists Alternative recommendations are given so the hospital physicists can choose methods that correspond to their experiences The recommendations include absolute calibration in absorbed dose as well as relative measurements for determining isodose configurations The methods are based on the calibration service of the British National Physics Laboratories, but are so general as to be easily applied by centres using other calibration services

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Vol 12 Fasc 3 | THERAPY PHYSICS BIOLOGY | 1973 June

CARCINOMA OF THE LIP

A series of 869 cases

K JØRGENSEN, O ELBRØND and A P ANDERSEN

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As the participants of this conference are actively working specialists in the field, the proceedings are a really up-to-date review of the present stage of development of computers in radiation therapy It can be recommended as a general introduction to the whole problem and as a comprehensive survey of more specialized topics

T Moller

GUIDANCE ON THE ACTION TO BE TAKEN IN THE EVENT OF INTAKE OF RADIOACTIVE MATERIAL INTO THE BODY HPA Report Series No 2 A PRACTICAL GUIDE TO ELECTRON DOSIMETRY 5—35 MeV HPA Report Series No 4 The Hospital Physicists' Association, London 1971

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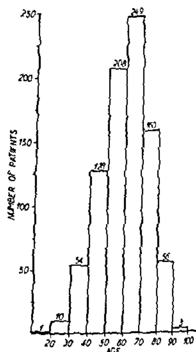


Fig 1 Age distribution at time of histology

ing 6 cases of basal cell carcinoma, the records in these stated that the growths seemed to have arisen from the epithelium of the vermilion border

The primary sign was a tumour, ulcer, or blister that was possibly crusting, the duration before their confirmation by histology was 0 to 4 months in 43 per cent, 4 to 8 months in 20 per cent, 8 to 12 months in 20 per cent, and more than 12 months in 17 per cent of the cases, an appreciable number of the cases thus had a relatively long history

Localization and classification Only those tumours arising from the vermilion area were included in the analysis, 853 (98 2 per cent) affected the lower lip and 16 (1 8 per cent) the upper lip

The series was classified in retrospect by the TNM system in the UICC formulation (UICC, Geneva 1968)

T1S pre invasive carcinoma, so called 'in situ'
T0 no evidence of primary tumour
T1 tumour measuring 2 cm or less in its largest dimension, strictly superficial or exophytic
T2 tumour measuring 2 cm or less in its largest dimension, with minimal infiltration in depth
T3 tumour measuring more than 2 cm in its largest dimension or one with deep infiltration, irrespective of its size

Table 1

The 869 cases of carcinoma of the lip grouped according to the TNM system (UICC)

	N0	N1	N2	N3	Total
T1	443	1	0	0	444
T2	337	1	0	1	339
T3	75	6	0	2	83
T4	0	0	1	2	3
Total	855	8	1	5	869

T4 tumour involving bone
N0 no palpable nodes
N1 movable homolateral nodes
N2 movable contralateral or bilateral nodes
N3 fixed nodes
M0 no evidence of distant metastases
M1 distant metastases

The rules thus fail to give a sharp distinction to the T groups As a criterion of distinguishing between T2 and T3 infiltration 5 mm in depth was chosen The classification was based upon fairly detailed descriptions in the records and by clinical photographs

Table 1 indicates the distribution within the TNM groups. All the cases were M0 A notable preponderance of cases in the early stages is apparent with only 14 N1, N2, and N3 cases, or 1 6 per cent (14/869) The records contained no information regarding the aetiology

Treatment The primary treatment was radium intubation in 766 of the 869 cases The principle consisted in puncture of the part of the lip involved transversely and longitudinally with 10 mg radium needles varying in number with the extent of the growth, the active length of the needles was 10 mm The needles were interspaced at 5 mm but in order to secure the dose at the margins of the neoplasm, the corresponding needles were placed in healthy tissue 5 mm from its macroscopic border The needles were usually in situ for four hours to yield a dose halfway between two needles ranging from 2 500 to 5 600 rad

Seventy four cases received primary external roentgen irradiation, 48 with a single large dose at 26 kV Fifteen cases were treated by fractionated [illegible] [illegible] supplementary to external irradiation One case had combined treatment in the form of low voltage irradiation and concomitant wedge-

Table 2

Frequency of recurrences and residual tumours in the entire series with primary treatment

	No. of cases	Residues and first recurrence	
		No.	Per cent
Radium intubation	766	84	11
26 kV 5 000 R or 5 100 R at one sitting	38	3	8
Other radiation therapy	36	11	31
Surgical treatment	29	1	1

shaped excision. Twenty-nine cases underwent primary operation, consisting in wedge-shaped excision in 27 cases combined with an Estlander plastic operation in one case while the entire prolabium was excised in another case.

Table 2 represents the frequency of recurrence and residues in relation to the primary therapeutic principle. Residues are taken to mean remnants of the neoplasm within the first two months of the primary treatment. Recurrence signifies a malignant process arising more than 2 months after the treatment, and less than 10 mm from the primary lesion. A fresh primary, on the other hand, is a malignant lesion at a distance of over 10 mm.

The groups in Table 2 are not intercomparable, but the table perhaps affords

Table 3

Grouping by therapeutic method of 165 cases of recurrences, residues and fresh primary lesions

Irradiation	
Lip intubation	82
26 kV at one sitting	4
Other radical irradiation	4
Palliative irradiation	3
Surgery	
Wedge shaped resection	51
Estlander type operation	9
Bernard type operation	5
Diefenbach type operation	2
Other types of resection	3
No treatment	2
Total	165

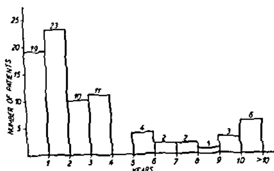


Fig 2 Time of occurrence of 81 local first recurrences

some impression of the level of the recurrence rate Fig 2 illustrates the time at which local recurrences arose Most appeared within the first 2 years It is remarkable that quite a few occurred after the 6th year and some more than 10 years after the primary treatment

A number of the patients, however, had more than one recurrence while several developed fresh primaries Of the 81 patients with one local recurrence 19 also had 2, 4 had 3, one had 4 and one patient had 5 local recurrences A total of 106 local recurrences occurred in these 81 patients Eighteen patients had 20 residues, 33 patients developed 39 fresh primaries Thus, in 132 cases, there were 165 local recurrences, residues, and fresh growths Table 3 indicates how these 165 cases were treated, that is generally either by radium intubation or wedge resection The most interesting thing about these figures is that by utilizing every therapeutic possibility it proved possible to control the primary lesion in all but 7 cases, of these, 5 cases also had regional lymph node metastases

The frequency of recurrence and residues in the group treated by radium intubation is related to the T classification (Table 4) As might be expected, with radium intubation of the lesions with deep infiltration, i e in the T3 group, the

Table 4

Frequency of residues and first recurrences after primary treatment with radium intubation in 766 cases grouped according to the T classification

	No of cases	No affected	Per cent
T1	378	28	7 4
T2	331	42	12 7
T3	57	14	24 6
T4	0	0	—

Table 5

Complications of treatment of local lesions

After radium intubation and external irradiation	
Cicatricial changes and tissue loss	17
Chronic ulceration treated by wedge excision	9
Chronic ulceration, untreated	5
Necrosis of bone	2
After surgery	
Microstoma	8
Wound rupture	1
Severe local infection (contributory cause of death on the 5th day in one case)	5
Total	43

recurrence rate was high, viz almost 25 per cent In such cases, therefore, it is wise to choose other forms of primary treatment

Complications All complications are included even if they are only just mentioned in the records, regardless of their severity As a whole, the rate was low (Table 5). Of 37 patients who received two radium intubations in the same area 7 had disturbing sequelae 4 developed a chronic ulcer which had to be excised, 1 recurrent ulceration and 2 patients fairly marked, disfiguring radiation-induced changes Of 3 patients who received a third intubation in the same area one is suffering from intermittent ulceration, one from chronic ulceration and one developed a further recurrence, treated surgically These values are low but it seems beyond doubt that the complication level is higher after re-intubation, in particular as regards disturbing complications that require treatment

Radium intubation affords satisfactory therapeutic results in cases with small, not deeply infiltrating tumours, mainly T1 and T2 Re-intubation should not be used for treating recurrences following primary radium intubation, as this involves a considerable risk of complications such as chronic ulceration requiring treatment, such cases necessarily demand secondary surgery

The present material fails to permit of conclusions regarding external roentgen irradiation, this was administered by a varying technique, and the results are scarcely comparable with the others

Metastases The prognosis of primary, localized carcinoma of the lip would appear to be excellent and could be controlled in all but 7 cases of the present material

The degree of malignancy of this condition is determined first and foremost by its tendency to produce metastases partly to the regional lymph nodes and partly

Table 6

Localization of presumed and confirmed regional lymph node metastases, primary and secondary, in 59 cases

Submental	Submandibular	Submental +submandibular	Submental +submandibular+ sup jugular	Submandibular+ inf jugular	Sup jugular
3	38 3 contralateral	8 1 bilateral	8 1 bilateral	1	1 Boeck's disease

beyond this barrier Distant metastases were extremely rare and occurred in only 5 cases The prognosis depends on the whole primarily on the treatment of regional lymph node metastases in the neck

The frequency of regional nodal metastases confirmed or presumed in this series of 869 cases with primary metastases was 1 6 per cent (14/869) and with secondary metastases 5 2 per cent (45/869), i e rather low

The localizations of presumed and confirmed regional lymph node metastases, primary as well as secondary, appear in Table 6 Most involved the submandibular nodes with only a few in the submental nodes alone Doubtful bilateral or contralateral nodes were present in 5 cases Only one case had palpable superior jugular nodes without simultaneously questionable submandibular or submental nodes, histology however proved this to be a case of Boeck's sarcoid It must be said therefore that in this series the regional metastases have accurately followed the paths of lymph drainage from the lips without missing any 'stations'

It would be of interest to find a group or groups particularly exposed to developing metastases in the cervical lymph nodes, and in which special measures might be considered, e g follow-up at shorter intervals than usual or possibly prophylactic neck dissections

The age distribution in the group with metastases, related to that in the entire series, perhaps represented a somewhat greater tendency to embrace metastases in the 70 to 80 year group, although this failed to amount to a definite bias

By far the largest number of metastases occurred within the first 30 months after the primary tumour was diagnosed, most being detected either at the first examination (14 cases) or at subsequent controls (28 cases) Seventeen patients had themselves noticed the metastases Five of the [illegible] ier advice

[illegible] series by the [illegible], are presented in Fig 3 This indicates a distinct bias, the metastatic rate being, as expected, highest in the T3 and T4 groups (100 per cent

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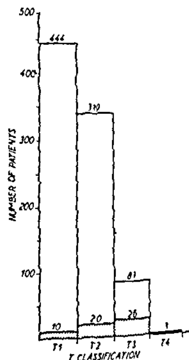


Fig. 3. T classification of the entire series and of 59 cases with metastases.

in T4), metastases also occurred however in the T1 and T2 groups.

The secondary metastatic rates, partly in the group without recurrence of the primary tumour and partly in that with recurrence, residues, or further lesion, related to the T classification, appear in Table 7. The secondary metastatic rate in the latter group of patients reveals that about 38 per cent in the T3 group developed metastases.

Treatment of regional lymph node metastases. Thirty-seven cases were treated by surgery and 22 by radiation therapy, with curative intention in 10, and as a palliative measure in 12 cases (Table 8). The two groups are not comparable, inter alia, because a relatively far larger number of cases, 18 and 12 respectively, in the irradiated group than in the surgically treated group, had fixed nodes. Five of the irradiated patients were alive at five years (from the diagnosis of the primary tumour) although a further 3 died of the condition later. One patient is alive with recurrence at five years. Thus, within this group only one patient is living without signs of recurrence.

Thirty-seven patients were treated by surgery, 25 (68 per cent) were alive at 5 years, but in 10 of these no signs of carcinoma in the lymph nodes were revealed at microscopy; if these cases be excluded, the 5-year survival rate becomes 56 per cent (15/27) in the group with confirmed lymph node metastases. The nature and number of operations appear in Table 9. This also gives the 5-year survival

Table 7

Frequency of secondary metastases in relation to T classification and occurrence of local recurrence, residues or fresh primaries

	No recurrence of primary tumour	Frequency of secondary metastases	Number of local recurrences, residues or fresh primaries	Frequency of secondary metastases
T1	389	6 (1.6 %)	54	3 (6 %)
T2	287	9 (3.1 %)	50	9 (18 %)
T3	54	10 (18.5 %)	21	8 (38 %)
T4	0	0	0	0
Total	730	25 (3.4 %)	125	20 (16 %)

Table 8

Grouping, by method of treatment, of 59 cases with presumed and confirmed regional lymph node metastases

	No	Died of carcinoma of the lip	Died of other causes	Alive at end of 5 years	Died of carcinoma of the lip after 5 years
Surgical treatment	37	9	3	25 (68 %)	1
Irradiation	22	16	1	5* (23 %)	3
Total	59	25	4	30 (51 %)	4

* 1 patient alive with recurrence at 5 years

Table 9

Grouping of 27 cases with lymph node metastases by nature of treatment

Nature of operation	No of cases	No of recurrences	5 year survival		
			After first operation	After operation for recurrence	Total
Radical neck dissection	8	3/8	4/8	0/2	4/8
Bilateral neck dissection	4	2/4	1/4	1/1	2/4
Partial neck dissection	15	9/15	5/15	4/15	9/15
Total	27	14/27	10/27	5/8	15/27 56 %

of the 27 cases with positive histology grouped by the nature of the surgery, the recurrence rate was relatively high following partial neck dissections A retrospective analysis of the recurrences in this group fails to elucidate whether the rate would have been lower if the surgical procedures had been more radical although there is reason to believe that it would have been

The prognosis depends upon whether the nodal metastases were movable or fixed Only 2 of the 25 patients with the former died of carcinoma of the lip whereas death occurred in 8 of the 12 with fixed nodes Invasion by the neoplasm into or fixation of the lymph nodes to the mandible spells a particularly poor prognosis All such patients (a total of 22) died

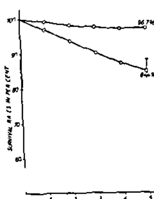
Cardiac arrest at 24 hours constituted a postoperative complication in a patient with ischaemic heart disease As for other sequelae it may be mentioned that one patient developed vagal paralysis and another peripheral palsy of the facial nerve In addition, a few cases of postoperative haemorrhage required secondary haemostasis

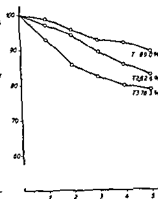
Most of the cervical metastases appear within the first 30 months, during this period therefore the patients should be seen at brief intervals and should be told to present themselves before their appointments if in doubt about anything The metastatic rate in the T4 group was 100 per cent The rate was then, as expected, highest in the T3 group and in the group with recurrence of the primary tumour, residue or a fresh primary Accordingly, these two groups should be followed with particular care The metastatic rate in the T3 group with any malignancy in the primary area is so high (in the present series about 38 per cent) that in this group it must be reasonable to perform prophylactic neck dissection simultaneously with the treatment of the first recurrence Suprahyoid and supraomohyoid neck dissections appear to be followed by a high recurrence rate and should, therefore, as far as possible be omitted in favour of radical neck dissections Invasion into the mandible is a serious prognostic sign and indicates a particularly extensive surgical procedure Irradiation of cervical node metastases in 22 cases (with a curative intention in 10) led to presumed cure in only one case

Special results The series included 24 women, the ratio for carcinoma of the upper lip being 5/24, or 21 per cent By comparison, it may be mentioned that the corresponding ratio for men was 1 3 per cent (11/845) This difference is significant Two of the cases affecting the upper lip of women were of basal cell carcinomas

Four of the 16 cases of carcinoma of the upper lip were basal cell carcinomas Prognostically, this group failed to differ from the entire series, with only one death within the control period

Survival calculations The survival curve for the entire series is illustrated in Fig 4 The 5 year crude survival was 84 per cent with a 2 times standard devia-





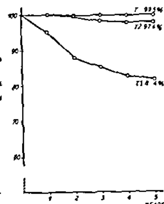


Fig 4 Fig 5 Fig 6

Fig 4 Survival curves for the entire series The bottom curve is uncorrected whereas the top curve is corrected for mortality from causes other than carcinoma of the lip

Fig 5 Uncorrected survival curves for T1, T2 and T3 cases

Fig 6 Survival curves for T1, T2 and T3 cases corrected for mortality from causes other than carcinoma of the lip

tion of only 2 5 per cent due to the large number of patients After correction for mortality from causes other than malignancy the 5-year survival became 96 7 per cent In other words, only 3 to 4 per cent of the patients succumbed to carcinoma of the lip Compared with other materials, these values are high but it must be borne in mind that the greater part of the series represents relatively early stages of the disease Fig 5 gives the 5 year survival curves for the T1, T2 and T3 groups After correction for causes of death other than carcinoma (Fig 6) the difference between T1 and T2 on the one hand and T3 on the other is more marked, it is mainly in the T3 group that the cancer deaths occur and, as already mentioned, is due to a higher tendency to lymph node metastases rather than to local factors per se

Discussion

A surprising variation in the sex ratio has been reported, from 2 per cent women in the series of MARTIN et coll (1941) to 28 per cent in that of PLAZA & AVELLO from Peru (1966) MALMIO (1967) reported 7 per cent (151/2138), and LYNCH (1968) in a series from Northern Ireland, 2 7 per cent In the present material only 2 8 per cent of the patients were women

The histology reports contained nothing of great interest Six cases of basal cell carcinoma were included Other authors (MARTIN et coll) have excluded

this condition in their belief that it cannot arise from the vermilion border, owing to the small number of cases in the present series this problem is however of little importance

The duration of symptoms and signs has varied within wide limits in the series reported MARTIN et coll gave an average of 15 months, KENNEDY (1934) 9 to 10 months and RATZKOWSKI et coll (1966) a little under 6 months. The mean duration of signs in the present series was around 6 months, an indication that the afflicted patients appeared early, indeed this is reflected in the staging of a relative preponderance of T1 and T2 cases

Poorly defined classification rules make a distinction between the T groups somewhat difficult The present authors selected 5 mm infiltration in depth as a distinction between T2 and T3, this proved easy to administer Quite often the records gave data such as "about 0 5 cm infiltration in depth", these cases were assigned to the T3 group It is thus apparent that the T3 group differs completely from the T2 group, both in respect to metastases and prognosis, a 5 mm criterion of distinction therefore seems reasonable

A few only of the series published have been TNM classified, in a large series of more than 2 000 patients, MAHONEY (1969) reported TNM distribution little different from those now presented

Only about 2 per cent of cases affected the upper lip, whereas an incidence of 5 to 10 per cent has been recorded in most other series

The present analysis cannot contribute much to the aetiology considerations but others have been able to demonstrate important factors EBENIUS (1943) in his thesis has suggested the importance of pipe smoking as an aetiologic factor BERNIER et coll (1951) demonstrated that exposure to sunlight as well as the degree of skin pigmentation are decisive factors in the production of carcinoma of the lip, LYNCH also discussed the aetiologic role of exposure to the sun

It is apparent from the account of local therapy that the tumour could be controlled in all but 7 cases, 5 of which had lymph node metastases Radium intubation produced satisfactory results, both from the curative as well as the cosmetic points of view However, the recurrence rate in the T3 group was high, 25 per cent, and this group should not be treated by radium implantation but better by fractionated roentgen irradiation or primary surgery The latter is the choice, if an operation is to be carried out simultaneously on the cervical nodes Roentgen irradiation with a low tube potential at a single sitting has afforded good results in the early stages, but has now been completely abandoned KAPATI et coll (1966) and RATZKOWSKI (1966) appeared satisfied with radium intubation, whereas others (STANLEY LEE & WILSON 1970, JESSE 1967) inclined to the view that fractionated roentgen irradiation is preferable No previous authors appear to have advocated primary surgical treatment

The frequency of lymph node metastases in the present series was low — only 5 6 per cent (49/869) Fairly high frequencies of metastases have been reported in early publications KENNEDY (1934) gave 24 per cent while from Canada (WATSON & BURKELL 1955) the figure was 8 5 per cent It appears to be widely agreed that neck dissection is the correct treatment, with most authors recommending standard radical neck dissection MAHONEY (1969) suggested that contralateral partial neck dissection be performed at the same stage

Several authors have advocated prophylactic neck dissection (FIGI 1932, KENNEDY 1934), many have taken an interest in this problem, and in recent years it has been widely agreed that this procedure was not justified, a view that the present authors of course share in general However, the fact that about 38 per cent of those cases of the T3 group that developed one or more local recurrences also had metastases of the cervical nodes suggests that this group should be subjected to cervical dissection

Conclusion

The material comprised 869 cases with histologically confirmed carcinoma of the lip primarily treated during the period 1946—1966 The cases were TNM classified The primary treatment consisted of radium intubation in 766 cases, the recurrence rate was low and the cosmetic results satisfactory However, the incidence of residues and recurrence was 25 per cent in the T3 tumour group, so that for this group primary surgery is suggested Although 132 patients had either residues, recurrences or else developed fresh primaries, the condition could be controlled in all but 7 patients, 5 of whom had lymph node metastases

Primary metastases in regional lymph nodes were present only in 14 cases (1 6 per cent) while 45 (5 2 per cent) had secondary metastases These appeared never to have missed primary lymph node stations Radiation therapy of the nodal metastases produced poor results, but after neck dissection the 5 year survival was 68 per cent (25/37) A transverse analysis indicated that of T3 cases recurring for the first time 38 per cent had, or later developed lymph node metastases Prophylactic neck dissection thus appears to be suggested in this particular group

The 5 year survival for the entire series was 84 5 per cent, and after correction for mortality from causes other than malignant disease, was 96 7 per cent, in other words, under 4 per cent of the patients died of the condition

SUMMARY

A total of 869 cases of carcinoma of the lip were reviewed The 5 year crude survival was 84.5 per cent Primary treatment consisted of radium intubation in most cases Recurrence

rate was low and the cosmetic results satisfactory For T3 tumour group primary surgery is suggested After neck dissection in cases of nodal metastases the 5 year crude survival became 56 per cent

ZUSAMMENFASSUNG

Insgesamt 869 Fälle von Lippenkarzinomen wurden behandelt Die gesamte 5 Jahres Überlebensrate betrug 84 5 Prozent Die primäre Behandlung bestand in einer Radium intubation in den meisten Fällen Die Rezidivfrequenz war niedrig und die kosmetischen Resultate zufriedenstellend Für die T3 Tumorgruppe wird die primäre chirurgische Behandlung vorgeschlagen Bei Fällen mit Lymphmetastasen betrug nach Halsdissektion die gesamte 5 Jahres Überlebensrate 56 Prozent

RÉSUMÉ

Les auteurs ont passé en revue un total de 869 cas de cancer de la levre Le taux brut de survie à 5 ans etait de 84 5 pour cent Le traitement primaire a consisté en implantation de radium dans la plupart des cas Le taux de récidive etait bas et les resultats esthetiques *satisfaisants Les auteurs proposent un traitement chirurgical primitif pour les tumeurs* du groupe T3 Après dissection cervicale dans les cas de metastases ganglionnaires le taux brut de survie à 5 ans est de 56 pour cent

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THE EFFECT OF ROENTGEN IRRADIATION ON MONOAMINE CONTAINING NEURONS IN THE RAT BRAIN

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Ionizing irradiation has often been used in the course of treatment of certain brain tumours The morphology and organization of the CNS neurons containing monoamine (MA) are well known (CARLSSON et coll 1962, DAHLSTROM & FUXE 1964, 1965, FUXE 1965, HILLARP et coll 1966) and may influence activities such as endocrine functions (FUXE & HOKFELT 1970) behaviour and emotions (SCHILDKRAUT & KETY 1967) It was therefore thought it might be of interest to investigate the effect of roentgen irradiation on central catecholamine (CA) and serotonine (5 HT) containing neurons Earlier investigations by PALAIC & SUPEK (1965, 1966) have indicated an effect of whole body irradiation on the content of noradrenaline (NA) and 5 HT in the mammalian brain with quantitative methods No morphologic investigation of the central MA neurons following roentgen irradiation appears so far to have been published The present work was therefore undertaken by the histochemical fluorescence method of Hillarp & Falck for the demonstration of tissue MA (for references and description see e g FALCK & OWMAN 1965, CORRODI & JONSSON 1967), in combination with quantitative biochemical procedures

Material and Methods Male albino rats of the Sprague-Dawley strain (200 to 250 g) were irradiated following nembutal, 25 to 30 mg/kg body weight i p

Submitted for publication 16 October 1972

(this dose induces light anaesthesia and keeps the animals immobile), the brain being approached from the ventral side with radiation of conventional quality (voltage 200 kV, filter 0 2 mm Cu, HVL 0 9 Cu), field area 2 cm × 3 cm salivary glands not included in the field Each animal received 4 000 R measured with an ionization chamber over 10 min and carefully watched during the exposure to control that it did not move Twenty-four hours, 4 or 7 days after irradiation the rats were killed Control animals were also anaesthetised with nembutal and sham irradiated 24 hours or 7 days before death

The rats were killed by decapitation The hypothalamus and salivary glands of 7 rats in each group (24 hours and 7 days) were rapidly dissected out, immediately frozen in propane cooled by liquid nitrogen, freeze dried and treated for fluorescence microscopy as previously described (DAHLSTRÖM & FUXE 1964, CORRODI & JONSSON 1967), (Hetovac freeze drier running at — 65° C) Fluorescence microphotographs were taken with Gevaert Scopix G green sensitive film with exposure times ranging between 35 and 45 seconds

For the biochemical part of the investigation the whole brains were dissected out, immersed in ice cooled perchloric acid, and assayed for NA and dopamine (DA) content by the HÄGGENDAL (1963) and CARLSSON & WALDECK (1958) method The salivary glands were also dissected out and assayed for NA content The tissues were sometimes frozen in dry ice and stored at — 70° C until assayed The results were expressed per organ and not per g tissue All rats in each experimental series were of the same size at the beginning of the experiment, but decreased in weight after the irradiation

Results

No NA or 5-HT containing nerve cell bodies were detected, in agreement with, e g ANDÉN et coll (1966) in 7 control hypothalamic pieces A few small, DA cell bodies with weak fluorescence intensity were located close to the third ventricle within the n arcuatus (Fig 1 a) (cf FUXE 1964)

Fig 1 Eminentia medialis (EM) from rat hypothalamus The ventral surface lies in the lower part of the figures (V) third ventricle × 115 a) Normal rat Two weakly green fluorescent DA cell bodies belonging to the tubero infundibular DA system (→) Strongly green fluorescent thin DA terminals close to the capillaries under the ventral surface (►) Green fluorescent NA terminals with thick varicosities under the third ventricle b) Roentgen irradiation at 24 h Strong diffuse green to yellow green background fluorescence Only a few nerve terminals evident dorsal to the EM with diffuse layer of DA terminals in most ventral part of EM (►), fluorescence lower than in normal animals Only few NA nerve terminals ventral to third ventricle c) Roentgen irradiation at 7 days Diffuse green to yellow green fluorescence in the hypothalamus at 24 h has disappeared The number of NA terminals in the brain tissue dorsal to the EM was lower than in normal rats Ventral to the third ventricle in the EM the visible NA terminals are more numerous than at 24 h with the thin green fluorescent DA terminals in the ventral part of the EM (►) less fluorescent than in the normal rat (a)

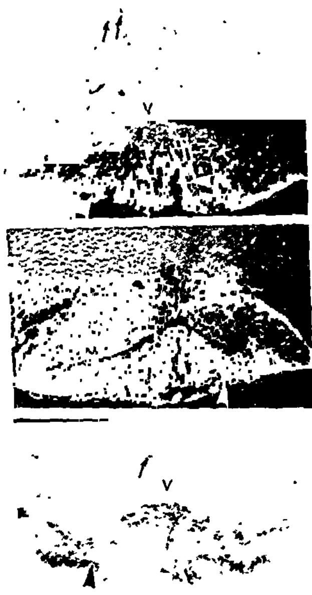


Fig 1 (For legend see opposite page)

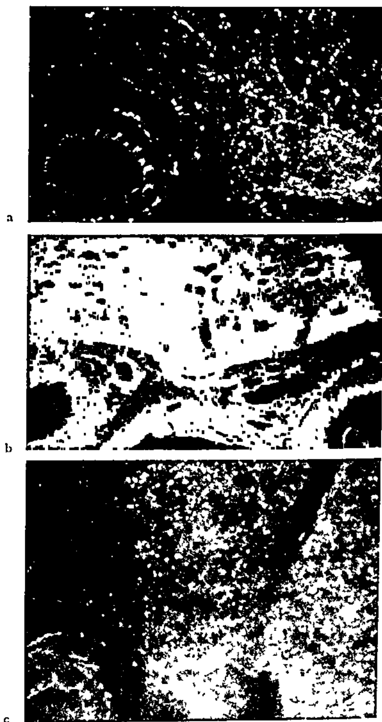


Fig 2. Lateral part (retrochiasmatic area) of ventral hypothalamus The ventral surface of the brain lies at the bottom ×180 a) Normal rat Large number of green fluorescent NA nerve terminals of different sizes Artery with NA nerve terminals in lamina adventitia (left) b) 24 h after roentgen irradiation Distinct appearance of the NA nerve terminals replaced by diffuse fluorescence in the tissue Unaffected vessel terminals originating from cell bodies in cervical superior ganglion c) Roentgen irradiation Seven days Distinct green fluorescent nerve terminals but fewer than in normal animals (a) Normal vessel terminals

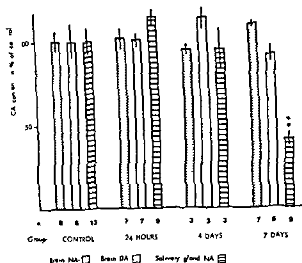


Fig 3 Content of brain and salivary gland catecholamines in the rat after irradiation Amounts given per organ and not per g of tissue and expressed as percentage of control values Mean ± SE indicated Number of observations (n) (bottom) ** significant decrease from control ($p < 0\ 005$)

The nerve terminals of all control rats were of varying diameter with a distinct outline The green fluorescence NA terminals had a strong fluorescence intensity (Fig 2 a) while the yellow fluorescent 5-HT terminals were generally smaller in diameter with a weaker fluorescence intensity In the eminentia medialis the thin DA nerve terminals of the outer layer were of medium to strong fluorescence intensity and distinct (Fig 1 a) In 4 out of 7 animals, irradiated 24 hours beforehand, the MA nerve terminals had lost their distinct outlines, and a diffuse green to yellow-green background fluorescence was observed (Figs 1 b, 2 b) A few nerve terminal varicosities were still evident but poorly defined These remaining varicosities were all of large diameter The nerve terminals in the other 3 animals were well defined, and the background fluorescence appeared to be only slightly increased above normal levels

Seven days after irradiation the background fluorescence was low, as in the controls, and the nerve terminals were distinct and of approximately normal fluorescence intensity (*Figs 1 c*, 2 c) However, the DA terminals in the outer layer of the eminentia medialis were in most animals of weak fluorescence intensity (Fig 1 c) The number of visible nerve terminals in some animals appeared to be reduced (Figs 1 c, 2 c), but in others the number was in the control range

The nerve terminals of the basal arteries were always of strong fluorescence intensity with distinct outlines Irradiation thus failed to change their appearances (Fig 2) The nerve terminals of the salivary glands, arising from the cervical superior ganglia like the vessel terminals were always distinct in outline However, in some rats the fluorescence intensity at 7 days appeared reduced

Biochemistry The total NA and DA content was 614 5 ± 20 77 ng (100 ± 6 15 per cent) and 1 024 8 ± 47 21 ng (100 0 ± 10 25 per cent) (n=8) respectively in the brain of control rats The total amine levels did not change significantly after irradiation (Fig 3), a possible decrease in total DA levels may have occurred at 7 days (Fig 3) The control value was 261 8 ± 21 20 ng per gland (100 ± 8 10 per cent) (n = 13) in salivary glands At 24 hours after irradiation some small increase was noticed, while a marked decrease to about 40 per cent of control was present at 7 days (n = 9) (cf Fig 3)

Behaviour No systematic investigation of behaviour has been carried out but some general impressions and observations may be mentioned Twenty four hours after irradiation the rats had lost about 5 to 10g of their original weight of 200 to 250g and had slight diarrhoea The food and water intake appeared to be normal A slight increase in aggressivity was occasionally noticed directed both towards the investigator and towards other rats On the second day the food and water intake were clearly reduced and ceased during the following days The rats that were killed on the seventh day after irradiation were therefore given injections of Aminosol Glucose (a mixture of amino acids and glucose) s c and saline i p twice a day from the second day to the day of sacrifice These animals had lost about 40 to 60g in weight at sacrifice Some rats from another series not given these injections died 5 to 6 days after irradiation with a weight reduction of 100 to 120g comparable to about 50 per cent of the original body weight

The body posture of the 7 day rats resembled that seen after reserpine treatment with curved backbone and extremities tucked tightly under the body When not handled they displayed bursts of supranormal activity with jerks towards the edge of the cage, and occasional aggressive outbursts towards the handler The gonades of these animals were invariably retracted within the abdomen No typical convulsions were observed

Discussion

Berg & Lindgren (1958) demonstrated histologically delayed irradiation lesions after one single exposure of 3 000 R to the brain of rabbits Since rats which have a much smaller volume of brain, were employed in the present material the correction factors given by Du Sault (1958) and Kirk (1972) produced the same effect of irradiation a figure of 4 000 R was therefore chosen for the present experiments

With the histochemical fluorescence method of Hillarp and Falck for investigation of tissue MA the fluorescent product of NA and DA is green while the product of 5 HT is yellow fluorescent Previous work with phar

macologic treatments demonstrated the site of NA and DA terminals (e g DAHLSTRÖM & FUXE 1965) The distribution in rat brain of NA DA and 5 HT nerve terminals has been earlier described in detail (FUXE 1965, HILLARP et coll 1966) The hypothalamic part was chosen because of its richness in MA terminals in the present work on the effect of irradiation on MA terminals

The total content of brain NA or DA failed to change significantly after irradiation Nevertheless serious damage of the MA nerve terminals probably usually occurred at 24 hours, since the histochemical examination revealed that the amines had diffused out from the terminals, yielding an increased green to yellow green background fluorescence (Figs 1 b 2 b) This clearly demonstrates the importance of performing histochemical and biochemical examinations together, the latter can only demonstrate net changes of total content of the amine, while the altered distribution within the tissue may be the essential factor

PALAIC & SUPEK (1966) observed a decrease in rat brain NA at 24 hours after whole body irradiation Such a decrease seems not to occur after irradiation of the head (Fig 3) However, the histochemical observation indicated that at this time the amines may be situated to a large extent extraneuronally (Figs 1 b 2 b) The reason for this diffusion from the nerve terminals may be a direct influence of the storage sites (the amine storage granules), as suggested by BRINKMAN 1962 [illegible]

[illegible] (1971) Enzymes responsible for the breakdown of extraneuronally located amines were also probably inhibited since otherwise an increased background fluorescence indicating the presence of diffused but unmetabolized amines would not have been evident

The increased background fluorescence observed in many animals at 24 hours had disappeared at 7 days and the nerve terminals appeared again with a distinct outline (Figs 1 c 2 c) This indicated that the irradiation damage was reversible The DA terminals in the eminentia medialis still however usually presented weak fluorescence intensity (Fig 1 c) in spite of a normal or somewhat reduced content of whole brain DA (Fig 3) The DA content of the eminentia medialis probably contributes little to the total amount of brain DA, and any changes in only these DA terminals are not likely to be reflected in total brain DA levels

At 24 hours 4 out of 7 rats had definite histochemical signs of severed MA nerve terminals while 3 animals appeared normal all the 7 controls had normal MA terminals That 3 out of 7 rats in the 24 hour irradiation group appeared normal may have been due to individual variations in irradiation sensitivity or to the dose of 4 000 R this may possibly be in the border line of doses producing the type of damage observed

The rats ceased eating and drinking after the second day following irradiation Since only the head was irradiated, this is unlikely to have been due to any direct radiation damage of the intestines The animals never presented signs of hunger or thirst when attempts to feed and water them were made, the specific centres for these functions in the hypothalamus were possibly damaged by irradiation Since a clear effect of the MA neurons of most animals was evident in this investigation, the cessation of food and water intake might possibly be connected at least partly, to the changed appearances of these neuron systems It may be mentioned that rats given a large dose of reserpine 12 to 18 hours beforehand and having practically no monoamine transmission, refused to eat or drink within this period

The sensitivity for irradiation of the *peripheral NA nerve terminals* appeared by microscopy to be less than that of the central NA terminals The NA nerve terminals in the walls of the basal arteries of the brain, terminals that originated from cell bodies in the cervical superior ganglion, always had a normal, distinct outline while the NA terminals in the brain near the vessels were seriously damaged (Fig 2)

The NA content of the salivary glands, adrenergically innervated by the cervical superior ganglion, had however decreased quantitatively after 7 days (Fig 3) The terminals of the basal arteries of the brain may well have a decreased NA content in spite of the normal histochemical appearances since the concentration of NA in the varicosities is normally so high that quenching of the fluorescence occurs (e g RITZÉN 1966) A marked decrease in the concentrations may therefore occur without detection in the ordinary fluorescence microscope, a somewhat decreased fluorescence intensity was observed in the salivary gland nerve terminals of some of the 7 day rats Alternatively, there may be a difference in the response between salivary gland and vessel terminals

This investigation has demonstrated that roentgen irradiation of the brain may injure central MA terminals Even if no true convulsions were observed in the present work the fact that reduced MA transmission may lower the threshold for epileptic seizures (HOLLISTER 1964), may indicate that seizures after therapeutic irradiation of the brain may possibly be related to impaired MA transmission

Acknowledgements

The present investigation was supported by grants from the Swedish Medical Research Council, Stiftelsen Magnus Bergwall Foundation The Research Foundation of Jubileums kliniken and the Medical Faculty University of Gothenburg (Gustav and Majet Lindgrens Foundation) The authors are indebted for technical assistance to Mrs Lillemor Berry and Mrs Kirsten Collin as well as to Mr Per Anders Larsson who also prepared the photographs and to Miss Mildred Pettersson

SUMMARY

[illegible]
no clear histochemical differences

ZUSAMMENFASSUNG

Der Effekt einer lokalen Bestrahlung mit 4 000 R des Kopfes auf die zentralen monoaminergischen Neuronen des Rattengehirns wurde histochemisch und biochemisch durch Fluoreszensmessungen untersucht Ein Strahlenschaden war lediglich durch histochemische Methoden feststellbar, was zeigt, dass der Gesamtgehalt des Gehirns an Monoaminen trotz der Leckage der Amine von deren Begrenzungen im wesentlichen unverandert war Die Speicheldrusen zeigten einen markanten Abfall im Gesamt Noradrenalingehalt nach 7 Tagen, jedoch keine klare histochemische Differenzen

RÉSUMÉ

Les auteurs ont etudie par des methodes histochimique et biochimique de fluoroscence l effet d une irradiation locale de 4 000 R de la tête sur les neurones mono aminergiques centraux du cerveau de rat La perturbation due à l irradiation n'a ete decelee que par les methodes histochimiques, montrant que le contenu total du cerveau en mono amines peut rester essentiellement inchange malgre la fuite des amines hors des terminaisons Le NA total a diminue de façon importante dans les glandes salivaires pendant 7 jours mais il n'y avait de differences histochimiques nettes

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LATE EFFECTS OF IRRADIATION ON THE THYROID GLAND IN MICE

III Comparison between irradiation of foetuses and adults

GUNNAR WALINDER and ANNE MARIE SJODEN

Late effects of ^{131}I and roentgen irradiation on the thyroid gland of the CBA mouse have been previously investigated after exposing adult animals (WALINDER 1972 b) and foetuses (WALINDER & SJODEN 1972) to various radiation doses It was not possible, however, to make any direct comparisons between the results of the two experiments since they were carried out at different times and because a substantial number of the animals that were irradiated at the foetal stage died before the age of 2 years

These complications were avoided in the present investigation by studying mice that were born at the same time Some of the animals were irradiated at the foetal stage and others when they had reached the age of 3 months Furthermore, the animals were killed already at one year of age, i e at a time before which very few mice had died

Material and Methods

Males and females of the CBA mouse strain were used The animals were born within the course of one week The foetuses were exposed to radiation by intra

Submitted for publication 22 September 1972

Table

Thyroid and pituitary weights and number of thyroid neoplasms in mice exposed to ^{131}I, roentgen rays, or a combination of the two types of radiation P denotes the significance of the deviations from the glandular weights in non irradiated mice The roentgen dose given in the table is the mean dose to the central parts of the pregnant mothers

^{131}I dose to central thyroid lobe, rad	Roentgen dose, rad	No of mice	Sex	Thyroid weight, mg (±SE)	p	Pituitary weight, mg (±SE)	p	No of adenomas	No of carcinomas
Mice irradiated in utero									
—	—	53	M	13 8±0 2		1 94±0 04		0	0
—	—	62	F	12 3±0 2		2 14±0 05		0	0
2400	—	14	M	13 0±0 6	—	2 09±0 09	—	0	0
2400	—	5	F	11 9±1 3	—	2 26±0 09	—	0	0
4700	—	53	M	11 2±0 2	< 0 001	2 13±0 03	< 0 001	0	0
4700	—	48	F	9 3±0 2	< 0 001	2 24±0 05	—	0	0
7800	—	58	M	7 8±0 2	< 0 001	2 21±0 04	< 0 001	3	2
7800	—	51	F	6 9±0 2	< 0 001	2 47±0 05	< 0 001	0	2
—	180	47	M	14 2±0 3	—	1 85±0 05	—	0	0
—	180	35	F	11 7±0 2	—	2 05±0 05	—	0	0
2400	180	5	M	11 7±0 6	< 0 01	1 66±0 09	< 0 05	0	0
2400	180	4	F	10 6±1 2	< 0 05	1 93±0 26	—	0	0
4700	180	45	M	9 1±0 3	< 0 001	1 99±0 04	—	0	0
4700	180	29	F	8 4±0 2	< 0 001	2 03±0 05	—	2	0
Mice irradiated as adults									
8500	—	45	M	9 0±0 2	< 0 001	2 02±0 04	—	0	0
9500	—	46	F	6 7±0 2	< 0 001	2 11±0 04	—	0	0

venous injections of ^{131}I solutions into the mothers or whole body roentgen irradiation on the 18th day of gestation, i e usually 2 days before the birth of the animals The adult mice were irradiated by intraperitoneal injections of ^{131}I on their 96th day of life The mice irradiated as foetuses were killed at 323 to 348 days of age and those irradiated as adults at 374 to 376 days of age

The housing and management of the mice were the same as described earlier The doses from ^{131}I to the thyroid glands were determined according to WALINDER (1971 and 1972 a) The whole body irradiations of the pregnant mothers with roentgen rays were carried out with the animals placed in a slowly rotating

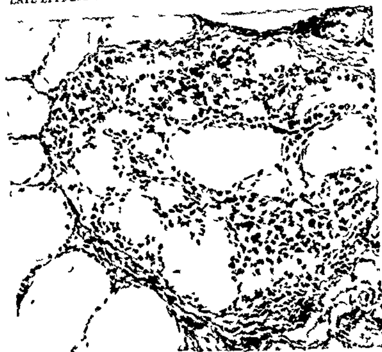


Fig 1 The most common type of benign tumour observed in the irradiated thyroid gland, mixed micro- and macro-follicular adenoma (van Gieson × 300)

plexiglass wheel at a distance of 45 cm from the anode of the roentgen tube The average dose rate to the 'midline' of the pregnant mice was 68 rad/min The [illegible] orm according to the [illegible] atter conditions The [illegible] was 58 rad/min, i e [illegible] between *maximum* and minimum doses was 1 3 The exposure time was 2 65 minutes giving a *total* dose *to the* central parts of the pregnant mothers of 180 rad The *roentgen radiation* data were identical with *those described in* paper II

The techniques used for sectioning, *fixation*, and staining of the thyroid and pituitary tissues have been described earlier *in paper I*

The criteria applied *for* distinguishing adenomatous goitre nodules from true adenomas and the classification of benign and malignant neoplasias were identical with the corresponding *definitions* in the two previous papers



a



b

Fig. 2. Follicular carcinoma. a) Two follicle cells showing advanced atypical nuclei with concentration of chromatin. Some mitotic figures can be seen. b) Neoplastic tissue from the same tumour invading (from the right) a parathyroid gland. (van Gieson × 300)



Fig 3 Papillary carcinoma Characteristic features of this type of tumour Papillae with prominent connective tissue stalks pale nuclei irregular aggregation of cells and often (as in this case) abundant mitotic figures (van Gieson × 300)

Results

With the exception of some losses during the suckling period there were few premature deaths among the animals in this investigation (3 females exposed to 7 800 rad, 2 males exposed to roentgen rays, and 1 male and 1 female exposed to ^{131}I as adults) No significant difference in the size of the litters between irradiated and non irradiated foetuses was detected

The different ^{131}I doses had no effect on the body weights The roentgen irradiation decreased the mean body weight by 8 %

The effect of irradiation on the weights of the thyroid and pituitary glands, as well as the number of tumours in the thyroids, are shown in the Table There was a significant inhibition of the age dependent thyroid growth in the about 1 year-old mice that had been irradiated as foetuses with a mean ^{131}I dose of 4 700 rad to the central parts of the thyroid glands and in those that had received 180 rad roentgen radiation in combination with a ^{131}I dose exceeding 2 400 rad in the central lobes

The weights of the pituitary glands increased with increasing doses to the thyroid in animals that had been exposed in utero solely to ^{131}I This was not the case in roentgen irradiated mice nor in animals that had been irradiated at 96 days of age No hypophyseal adenomas were observed

Three benign and four malignant tumours were found in the thyroid glands of mice that had been exposed as foetuses to 7 800 rad from ^{131}I and 2 thyroid adenomas were found among the animals that had received 180 rad roentgen radiation in combination with 4 700 rad from ^{131}I in utero No thyroid tumours could be seen in any of the other animal groups In spite of the high radiation doses to the thyroids in the mice irradiated at 96 days of age no tumours could be found in their glands

Discussion

The relationship between radiation doses to the thyroid and the degree of growth inhibition of the gland was similar to that observed in the previous investigation (paper II) The positive correlation between radiation doses to the thyroids and the pituitary weights observed in the ^{131}I irradiated animals could not be seen in mice exposed to a combination of ^{131}I and whole body irradiation with roentgen rays Despite the substantial shrinkage of the thyroid tissue in the latter cases, the roentgen irradiated pituitary glands could obviously not respond to this growth challenge, as was the case with the non-irradiated hypophyses This appears to indicate an even higher radiosensitivity of the hypophyseal epithelium than that of the thyroidal cells This difference might, however, be an apparent one since changes in the number of cells in the pituitary are immediately reflected in a corresponding change in the weight of the gland, which is usually not the case in the thyroid

There were seven thyroid tumours in *109* mice exposed on the *18th day of* gestation to 7 800 rad from ^{131}I to the central parts of the glandular lobes No tumours were found in animals irradiated as adults If we assume that a) the high doses to the adult animals had not seriously affected the neoplastic development of the initial radiation damage and b) the occurrence of tumours is binomially distributed, we find, *by applying the 'null hypothesis', that the mouse foetus is* more susceptible to radiation induced thyroid neoplasia than the *adult animal* ($p = 0\ 013$) Since the thyroid glands in animals that had been irradiated as adults were not smaller (and consistently not more atrophic) than those irradiated with 7 800 rad from ^{131}I when in utero, the point (a) above does not *contradict the conclusion that the foetal thyroid gland is more radiosensitive* than that of the adult mouse

In the earlier investigation the irradiated mouse foetuses were examined when they had reached the age of 2 years The tumour frequency in the thyroid glands was then very high in mice that had been exposed to 4 700—4 900 rad in the central parts of the lobes (31 % in males and 25 % in females) These figures were approximately equal to those found in mice that had been exposed to 6 800

—7 300 rad. The present investigation indicates that although the eventual tumour frequency in the thyroids might be similar in the two groups of mice, the tumours will appear earlier in those mice that had received the higher doses. The high fequency of thyroid tumours in the irradiated 2-year-old mice is probably to a great extent a consequence of the marked thyroidal growth in CBA mice between their first and second year of life (WALINDER et coll. 1971).

The number of benign thyroid tumours is similar to that of malignant ones (2.8 % and 3.7 % respectively) in the 1-year-old mice exposed to 7 800 rad, whereas the percentage of benign tumours found in the previous investigation of 2-year-old mice exposed to 6 800 to 7 300 rad was 22 against 6 % for the malignant neoplasms. These figures indicate that the thyroid carcinomas in most instances did not develop in previously benign tumours.

Acknowledgement

This investigation was carried out as part of the programme of the European Late Effects Project Group (EULEP).

SUMMARY

The thyroid glands of mice born within the course of one week were irradiated a) at the foetal stage, by intravenous injections of ^{131}I solutions to the mothers on the 18th day of gestation, by whole body roentgen exposure, or by a combination of the two forms of radiation or b) as adults, by intraperitoneal injections of ^{131}I. In 109 1-year-old mice exposed in utero to 7 800 rad from ^{131}I (to the central parts of the glandular lobes), three benign and four malignant thyroid tumours were observed. No thyroid tumours could be found in 91 1-year-old animals exposed to 8 500 to 9 500 rad from ^{131}I at 3 months of age.

ZUSAMMENFASSUNG

Die Thyreoidea von Mäusen, die innerhalb einer Woche geboren waren, wurden a) während des Foetalstadiums durch intravenose Injektion von ^{131}I-Lösungen in das Muttertier am 18. Gestationstag, durch Gesamtkörperbestrahlung oder durch eine Kombination dieser beiden Bestrahlungsformen oder b) im Erwachsenenstadium durch intraperitoneale Injektion von ^{131}I bestrahlt. Bei 109 1 Jahr alten Mäusen, die in utero mit 7 800 rad von ^{131}I (die zentralen Teile des Thyreoidealappen) bestrahlt worden waren, fanden sich drei benigne und vier maligne Thyreoideatumoren. Bei 91 1 Jahr alten Tieren, die im Alter von 3 Monaten mit 8 500—9 500 rad von ^{131}I bestrahlt worden waren, konnten keine Tumoren gefunden werden.

RÉSUMÉ

La glande thyroïde de souris nées en une semaine a été irradiée a) au stade fœtal par injection intraveineuse de solution de ^{131}I aux mères au 18e jour de la gestation, par irradiation roentgen totale du corps, ou par une combinaison de ces deux formes de radiations ou

b) quand elles étaient adultes par injections intrapéritonéales de ^{131}I Sur les 109 souris âgées d'un an exposées in utero à 7 800 rad de ^{131}I (dose dans les parties centrales des lobes glandulaires) les auteurs ont observé trois tumeurs benignes et quatre tumeurs malignes de la thyroïde Ils n'ont pas trouvé de tumeur thyroidienne sur les 91 animaux âgées d un an exposes à 8 500—9 500 rad de ^{131}I à l'âge de trois mois

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INFLUENCE OF OESTROGENIC HORMONES ON CARCINOGENESIS AND TOXICITY OF RADIOSTRONTIUM

A. NILSSON and C. RONNBACK

Oestrogenic hormones given after sublethal irradiations bring about a striking increase of mortality rate in mice (THOMPSON et coll 1965, TREADWELL et coll 1943). Given before irradiation, however, they are said to be protective (ROOKS & DORFMAN 1961). In the intact animal (DOUGHERTY 1952, KAPPAS & PALMER 1963) these hormones cause acute thymic involution, which, seemingly, severely impairs the immunologic capacity (THOMPSON et coll 1966). On the other lymphatic tissues their effects are minimal, variable, and appear to relate to the species investigated (DOUGHERTY 1952, KAPPAS & PALMER 1963).

Oestrogenic hormones also regularly cause a marked formation of new endosteal bone in mice (GARDENER 1936, 1946, URIST et coll 1950, SIMMONS 1962). The proposed mechanism involves an increased formation of osteoblasts by transformation of reticular cells and primitive connective tissue cells from the bone marrow (SIMMONS 1962). There are also observations (MILLER et coll 1943) that oestrogen accelerates the development of osteosarcomas in strains of mice with spontaneous bone tumours. In a number of soft tissues such as the

Submitted for publication 13 June 1972

Table 1

Experiment I. Day 0. Start of experiment. All mice 75 ± 3 days old. Oestrogen was given as Estradurin

Group of mice	No. of mice	Day 0 oestrogen mg s.c.	Day 7 µCi ^{90}Sr/g body weight i.p.	Day 30 oestrogen mg s.c.	Day 60 oestrogen mg s.c.	Day 67 µCi ^{90}Sr/g body weight i.p.
A (males)	143	1.0	0.8	0.5	0.25	–
B "	118	–	0.8	–	–	–
C "	68	1.0	–	0.5	0.25	–
D "	50*	1.0	–	0.5	0.25	0.8
E (females)	50*	0.25	0.4	0.25	0.25	–
F "	100*	–	0.4	–	–	–

* One was lost during the course of the experiment.

mammary gland, testes and lymphoid tissues, pituitary and uterus oestrogens also exert a carcinogenic action in mice (GARDNER 1957).

Radiostrontium is a potent carcinogen. Given in doses which induce bone tumours it initially exerts a strongly suppressive effect on the bone cells. Later there is an increased activity and proliferation which ultimately leads to osteosarcoma induction (NILSSON 1962, 1970). It might be anticipated — since carcinogenesis requires cells capable of proliferation — that factors such as oestrogen, which stimulates these cell lines and their precursors to proliferation, will increase the carcinogenicity of ^{90}Sr.

The present investigation will primarily deal with the influence of oestrogen on the skeleton of ^{90}Sr-treated mice. Some combined effects on mortality and blood and blood-forming tissues are recorded but not systematically investigated.

Material and Methods

The material was divided into two main experiments. Male CBA mice (groups A, B, C and D) and female mice (groups E and F), all 75 ± 3 days old, were used. The mice, ten in each cage, were fed during the experiment on a standard diet ad libitum and kept in the same room under similar environmental conditions.

Experiment I. Six groups of mice were given $^{90}Sr(NO_3)_2$ intraperitoneally and in some cases also oestrogen of long duration (Estradurin, Leo) subcutaneously as recorded in Table 1. The mice were inspected twice a day during the whole experimental period. Before autopsy films were exposed of all dead mice

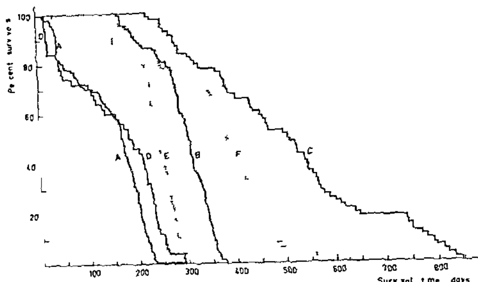


Fig 1 Survival time in days of different groups of mice after treatment For explanation of different group designation see Table 1

The diagnosis of bone tumours in this experiment is based entirely upon roentgenologic and post mortem findings, and thus the number of tumours recorded is a *minimum* Histologic classification of the bone tumours found was not performed, which means that tumours classified here as bone tumours are not necessarily *true bone tumours* Leukaemia and neoplasia in other tissues were, however, histologically verified Routinely the head was cut longitudinally through its midline and fixed The histologic methods applied to this material are identical with those used in experiment II

Experiment II These experiments were performed in order to investigate the histologic changes preceding tumour development and for classification of the tumours induced The experimental procedures are recorded in Table 2 Only male mice were used The mice were killed by cervical dislocation at different intervals as shown in Table 2 Before sacrifice the m [illegible]

[illegible] as the thymus, spleen and adrenal glands. These organs and both femora, tibiae, humeri, sternum, pelvic bones and the spine were fixed in Stieve's fluid The head was cut longitudinally through its midline

Table 2

Experiment II Day 0 Start of experiment All mice 75±3 days old Oestrogen was given as Estradurin

Group number	No of mice	Treatment			
		Day 0 oestrogen mg s c	Day 7 μCi ^{90}Sr/g body weight i p	Day 30 oestrogen mg s c	Day 60 oestrogen mg s c
1	58	1 0	0 8	0 5	0 25
2	14	1 0	—	0 5	0 25
3	25	—	0 8	—	—
4	10	—	—	—	—

and also fixed in Stieve's fluid The bones were decalcified in 20 % formic acid Ordinary histologic techniques were used and all sections were stained according to the van Gieson method and with haematoxylin and eosin Selected sections were also stained with PAS — orange G (head with tumours of the pituitary), azure-eosinate, azan according to Heidenhain, and Foot and Foot s silver method Mice spontaneously dead before sacrifice were autopsied and handled in the same way

Definition of tumour classification Bone tumours, when bone formation occurs, are called osteosarcomas These can be subdivided into fibroblastic, chondroblastic, osteoblastic or osteoclastic types depending upon whether the collagenous, cartilaginous, osseous or osteoclastic components predominates In addition pleomorphic, mixed and anaplastic types can be distinguished, depending upon whether a strongly pleomorphic cell type, equal parts of at least three cell components or a low differentiated tissue predominate This subdivision has been recommended by the Committee of Pathology of the European Late Effects Project Group (EULEP) (1971)

Results

Experiment I

Survival time From Fig 1 it is seen that mice given oestrogen + ^{90}Sr had a significantly shorter survival than mice given only ^{90}Sr The mean survival times are recorded in Table 3 During the first 3 months after the start of the experiment 45 mice (31 5 %) died in group A The mortality was concentrated to 3 consecutive periods of 2 to 3 weeks' duration following approximately 11 to 14

Table 2 *(cont)*

No of mice killed day after injection of ^{90}Sr and day after 1st injection of oestrogen (within brackets)										Number of mice dead before sacrifice
7 (14)	38 (45)	60 (67)	73 (80)	83 (90)	113 (120)	121 (128)	143 (150)	173 (180)	203 (210)	
2	2	5	2	5	5	2	5	5	5	20
2	2	—	2	4	—	2	2	—	—	0
—	—	5	—	—	5	—	—	5	5	5
—	—	2	—	3	—	2	—	—	3	0

days after the preceding treatment with either ^{90}Sr or oestrogen The highest death rate was seen after the second injection of oestrogen, when 25 mice (17 5 %) died In group D a similar pattern of mortality was observed after the ^{90}Sr administration in spite of the fact that ^{90}Sr was given seven days after the last hormone injection on day 60 Within 2 months 14 mice (28 %) died Also in group E some mice died very early as compared with mice treated with only ^{90}Sr or oestrogen

Causes of death Since there was a high initial death rate in mice given the combined treatment, the material was divided into two parts mice dying before (Table 4) and after the appearance of the first bone tumour (Table 5) Negative sections (Table 4) without gross anatomic or histologic changes which could unambiguously explain the cause of early death were numerous in group A and also in group D Possible explanations will be discussed later It is also seen that haemorrhage (usually haemothorax or haemocoelia) was a frequent cause of early death both in group A (27 %) and in group D (40 %)

In group A the first death in haemorrhage appeared 77 days after injection of ^{90}Sr or 17 days after the last oestrogen injection Between days 77 and 129, 12 cases occurred In group D all cases were found between days 17 and 120 after the injection of ^{90}Sr In this group all oestrogen injections were given before administration of ^{90}Sr In group E inanition in combination with a more or less insufficient haematopoiesis predominated

From Table 5 is evident that, in all groups except C, bone tumours predominated as cause of death In group E, however, many cases of bone tumours were complicated by a more or less severe pyometra, which in some cases made it difficult to settle the exact cause of death In group F a high death rate in

Table 2

Experiment II Day 0 Start of experiment All mice 75±3 days old Oestrogen was given as Estradurin

Group number	No of mice	Treatment			
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1	58	1 0	0 8	0 5	0 25
2	14	1 0	—	0 5	0 25
3	25	—	0 8	—	—
4	10	—	—	—	—

and also fixed in Stieve's fluid The bones were decalcified in 20 % formic acid Ordinary histologic techniques were used and all sections were stained according to the van Gieson method and with haematoxylin and eosin Selected sections were also stained with PAS — orange G (head with tumours of the pituitary), azure-eosinate, azan according to Heidenhain, and Foot and Foot s silver method Mice spontaneously dead before sacrifice were autopsied and handled in the same way

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Table 3 *(cont)*

B	C	D	E	F
118	68	50	49	99
293 4±2 9	502 1±21 2	157 9±12 3	229 3±9 5	368 1±10 4
5	0	20	6	5
173 2±1 3	—	58 3±9 8	73 7±20 8	158 4±6 5
189	—	159	179	184
316 1±1 1	—	224 4±5 8	251 0±4 1	379 3±9 7
99	0	29	42	75
14	68	0	1	19
264 (134)	—	133 (58)	175 (110)	186 (119)
264/113 = 2 3	—	133/29 = 4 6	175/43 = 4 1	186/94 = 2 0
264/118 = 2 2	—	133/49 = 2 7	175/49 = 3 6	186/99 = 1 9
168	256	42	250	170
209 8±14 9	371 9±32 4	122 4±35 0	262±4 9	223 9±8 2
9	14	5	4	17
7 6	20 6	10 2	8 2	17 2
—	7	—	—	—
—	620±65	—	—	—
	7	—	—	—
	486±67	—	—	—

Table 4

Causes of death in mice dying before the appearance of the first bone tumour

Group of mice	No of dead mice	Cause of death				
		Haemorrhage	Leukaemia	Inanition*	Neg autopsy	Not stated**
A	45	12	1	—	15	17
B	5	—	3	—	2	—
C	0	—	—	—	—	—
D	20	8	3	4	5	—
E	6	—	—	5	1	—
F	5	—	2	3	—	—

* These cases were combined with bone marrow hypoplasia

** On account of cadaverous changes and cannibalism

Table 3

Survival time, frequency of neoplasia and induction time

	Group of mice
	A
Number of mice	143
Mean survival, all mice, days	147 4±4 4
Number of mice dead before first bone tumour	45
Mean survival, mice dead before first bone tumour, days	51 0±3 9
Occurrence of first bone tumour, day after ^{90}Sr	127
Mean induction time, days	*191 1±2 6*
Number of *mice with bone tumours*	96
Number of mice without bone tumours	2
Total number of bone tumours (macroscopic within brackets)	414 (196)
Number of bone tumours/mouse	414/98 = 4 2
Number of bone tumours/mouse, whole material	414/143 = 2 9
Occurrence of first leukaemia, day after ^{90}Sr	76
Mean induction time, days	131 7±8 6
Number of mice with leukaemia	9
Percentage of leukaemia, whole material	6 3
Eosinophilic adenoma, pituitary	—
Mean induction time, pituitary adenoma, days	—
Other tumours	—
Mean induction time, other tumours, days	—

leukaemia was also noted In group C, besides a high frequency of peritonitis, there was also a high frequency of leukaemia (20 6 %) and other malignant neoplasm (10 3 %), such as carcinomas of the liver, leiomyosarcomas and fibrosarcomas in the peritoneal cavity Seven eosinophilic adenomas of the pituitary were also observed, out of which five (7 4 %) were the cause of death In this context a case of periarteritis nodosa of an abdominal vessel should also be mentioned, although not causing the death of the animal

Frequency of bone tumours The number of bone tumours per mouse, calculated from the whole material (intramedullary + overt tumours) in experiment I or only from overt tumours, was approximately a factor 2 greater in groups A and D compared to group B (Table 3, Fig 2) and in group E in relation to group F The induction time for these tumours was also significantly shorter ($p < 0.001$) *in all the groups treated with oestrogen and* ^{90}Sr as compared to mice treated with ^{90}Sr alone No bone tumours appeared in group C treated

Table 3 *(cont)*

B	C	D	E	F
118	68	50	49	99
293 4±2 9	502 1±21 2	157 9±12 3	229 3±9 5	368 1±10 4
5	0	20	6	5
173 2±1 3	—	58 3±9 8	73 7±20 8	158 4±6 5
189	—	159	179	184
316 1±1 1	—	224 4±5 8	251 0±4 1	379 3±9 7
99	0	29	42	75
14	68	0	1	19
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264/113 = 2 3	—	133/29 = 4 6	175/43 = 4 1	186/94 = 2 0
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168	256	42	250	170
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9	14	5	4	17
7 6	20 6	10 2	8 2	17 2
—	7	—	—	—
—	620±65	—	—	—
—	7	—	—	—
—	486±67	—	—	—

Table 4

Causes of death in mice dying before the appearance of the first bone tumour

Group of mice	No of dead mice	Cause of death				
		Haemorrhage	Leukaemia	Inanition*	Neg autopsy	Not stated**
A	45	12	1	—	15	17
B	5	—	3	—	2	—
C	0	—	—	—	—	—
D	20	8	3	4	5	—
E	6	—	—	5	1	—
F	5	—	2	3	—	—

* These cases were combined with bone marrow hypoplasia

** On account of cadaverous changes and cannibalism

Table 5

Causes of death in mice dying after appearance of first bone tumour in each group

Group of mice	No of mice	Cause of death							
		Bone tumours	Leukaemia	Not stated**	Inanition	Peritonitis	Haemorrhage	Neoplasia	Other causes
A	98	74	8	4	5	—	7	—	—
B	113	88	6	14	2	1	—	—	2
C	68	—	14	9	1	31	1	12	—
D	29	26	2	1	—	—	—	—	—
E	43	29*	4	2	1	1	—	—	6***
F	94	74	15	3	—	—	—	—	2

* In many cases pyometra also occurred
** On account of cadaverous changes and cannibalism
*** Pyometra

only with oestrogen in spite of a survival time approximately 3 3 times longer than for mice treated with both ^{90}Sr and oestrogen The occurrence of osteosarcomas is extremely rare in these mice

Site of tumours in the skeleton With respect to the anatomic distribution of the tumours there were only insignificant differences when oestrogen + ^{90}Sr treated mice were compared to those given ^{90}Sr alone, except for a lower frequency of tumours in the head in the former group

Incidence of leukaemia The frequency and latency time for leukaemia are recorded in Table 3 The leukaemia cases were subdivided into those starting as thymic lymphomas and those originating as bone marrow lymphomas, the percentage distribution of which is shown in Fig 3 The spontaneous incidence of leukaemia in this strain is in the order of 0 5 to 1 0 per cent

Experiment II

Histology Serial sections were performed in order to investigate presumptive differences in histology between mice treated with oestrogen, ^{90}Sr or oestrogen + ^{90}Sr A classification of bone tumours was also made (Table 6)

Mice treated with oestrogen In general the histology was in good agreement with that of previous observations (GARDNER 1946, URIST et coll 1950) The endosteal bone apposition is most obvious in the metaphysis of the distal femur, proximal tibiae and proximal humerus It was less marked in the sternum and intermediary in the pelvic bones and vertebrae On day 14 the trabeculae in the femoral metaphysis was somewhat longer and broader than normally At certain

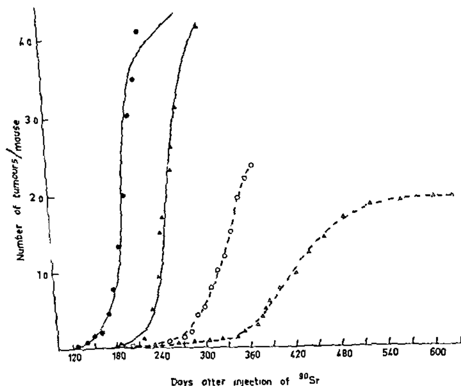


Fig 2 Number of tumours per mouse in relation to time after injection of ^{90}Sr ● group A, O group B, ▲ group E, △ group F *For explanation of different group designation see Table 1*

Table 6

Bone tumour classification serially killed mice experiment II

Treatment	No of tumours	Type of osteosarcoma, per cent				
		Osteoblastic	Fibroblastic	Osteoclastic	Mixed type	Pleomorphic
^{90}Sr + oestrogen	247	61 1	14 9	5 3	17 4	1 2
^{90}Sr	9	66 7	33 3	–	–	–

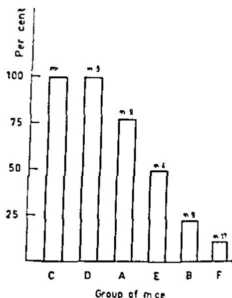


Fig 3 Percentage distribution of thymic lymphomas *in relation to non thymic (bone marrow)* lymphomas

places small clones of enlarged osteoblasts were detected The number of osteoclasts seems to have diminished In the sternum the bone formation was much less significant On day 45 about half of the femur from the distal metaphysis to the middle of the diaphysis was more or less filled with newly formed bone (Fig 4) The osteoblasts were still very active but were not so large as previously (Fig 5) Osteoclasts were still seen In the sternum the production of new bone occurred and the osteoblasts were numerous Osteoclasts were somewhat reduced in number

From day 67 to 150 after oestrogen injection the whole medullary cavity of the femur successively became more or less obliterated with new bone (Fig 6) The osteoblasts were small and quite inactive Osteoclasts were few

Mice treated with ^{90}Sr The histology does not differ from that of earlier descriptions (NILSSON 1962, 1970)

Mice treated with oestrogen + ^{90}Sr On day 14 after oestrogen injection the formation of bone in the femur was about the same as among mice treated only with oestrogen, but in addition there was a very marked formation of fibres and fusiform cell elements between and along the bone spiculae and endosteal linings Numerous enlarged osteoblasts were seen Osteoclasts were also numerous and many of them were located in typical Howship s lacunae In the sternum there was a very marked increase of active osteoblasts and osteoclasts and in



Fig 4

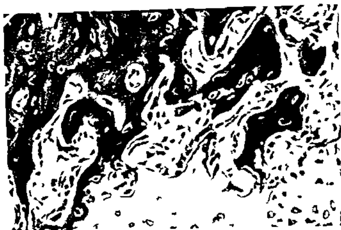


Fig 5

Fig 4 Femur male mouse 45 days after first injection of oestrogenic hormone Heavy formation of new bone filling up marrow cavity at distal end of the bone Van Gieson approx × 7

Fig 5 Magnification of distal metaphyseal part of the bone in Fig 4 Oestoblastic activity and newly formed bone Van Gieson × 200

some cases numerous mitoses were seen On day 45 the histologic examination of the femur differed remarkably from that in mice treated only with oestrogen (Fig 7) The occurrence of newly formed bone . . . between bone spiculae was filled with a q
The predominating cell type in this tiss
ments . . .
no t



Fig 6 Femur, male mouse, 121 days after first injection of oestrogen Almost complete occlusion of the marrow cavity by newly formed bone Van Gieson approx ×7

Fig 7 Femur, male mouse, 45 days after first injection of oestrogen and 38 days after injection of ^{90}Sr Compared with the bone in Fig 4 the formation of new bone is poor Almost complete aplasia of the marrow Van Gieson approx ×7

Fig 6 Fig 7

places there was an obvious osteoclastic activity In the sternum there was a great increase of very active osteoblasts, and the formation of bone was much greater than among mice given oestrogen alone Numerous osteoclasts were also seen From day 67 to 150 after the first oestrogen injection the difference between the two groups is even more accentuated In the femur the 'compactization' decreased with time since the newly formed 'oestrogenic' bone was broken down and to a great extent replaced by the formation of a fibrous tissue (Fig 9) In the sternum, on the other hand, the bone formation inside the medullary cavity was more intense than in the group treated with oestrogen alone No replacement



Fig 8 Magnification of Fig 7 Destruction of newly formed bone and formation of fuchsinophilic and argyrophilic fibres Some macrophages and fusiform cell elements but no normal osteoblasts are seen Aplastic fatty marrow Van Gieson × 175

of oestrogenic bone by a fibrous tissue component was observed in the sternum of mice treated with oestrogen + ^{90}Sr in contrast to that in the femur

It is inside these fibrous, originally quite cell-deficient tissues that small islands with morphologic characteristics of malignancy appeared These buds usually have an appearance of greater histologic activity and variation than was seen among mice treated with ^{90}Sr alone Of particular interest was the frequent occurrence of more or less pure osteoclastic osteosarcoma buds (Fig 10) Of importance also is the fact that the first microscopic osteosarcomas in the oestrogen + ^{90}Sr treated mice were detected histologically already on day 121 after the ^{90}Sr injection in both femur and spine, whereas the first microscopic tumours among mice treated with ^{90}Sr alone did not appear until after 173 days

Tumour frequency and histologic classification In the oestrogen + ^{90}Sr treated mice in experiment II altogether 247 osteosarcomas were found, of which 98 were macroscopically detectable For the whole material, 58 mice, there were 4 2 tumours/mouse This figure increases to 5 6 when only the 46 tumour bearing mice were taken into account In the ^{90}Sr group only 9 tumours were found, giving a mean of 0 4 tumours/mouse for 25 mice

The classification of tumours is recorded in Table 6 In the group treated with oestrogen and ^{90}Sr osteoblastic tumours predominated, followed by fibroblastic and mixed type, osteoclastic (Fig 11) and pleomorphic type osteosarcomas With reservation for the small number of tumours occurring among mice given only ^{90}Sr there was good agreement with earlier observations (NILSSON 1962)

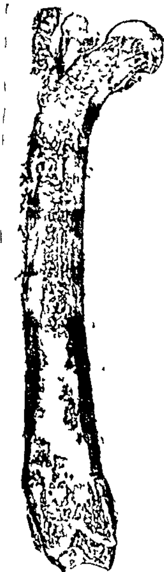


Fig 9

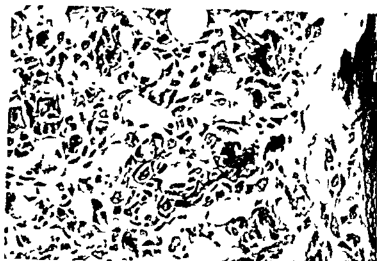


Fig 10

Fig 9 Femur male mouse, 121 days after first injection of oestrogen and 114 days after injection of ^{90}Sr In the diaphysis remnants of newly formed oestrogenic' bone Distally most of the oestrogenic bone destroyed and devitalized In the proximal part small growing osteosarcoma bud destroying preformed compact bone Van Gieson approx ×7

Fig 10 Magnification of proximal osteosarcoma bud with numerous osteoclast like and fusiform cell elements Van Gieson ×200

Blood and haematopoietic tissues The bone marrow depletion in the oestrogen + ^{90}Sr group was initially more severe than among the other groups, particularly in the femur On account of the earlier mentioned heavy formation of fibrous tissue of the marrow cavities in the femur, marrow regeneration was also strongly impaired Great differences were also seen in the sternal marrow, varying from an almost complete restoration of cellularity among the ^{90}Sr treated mice to a quantitatively strong impairment on account of heavy bone formation in the oestrogen + ^{90}Sr treated mice (Fig 12) In the mice treated with oestrogen

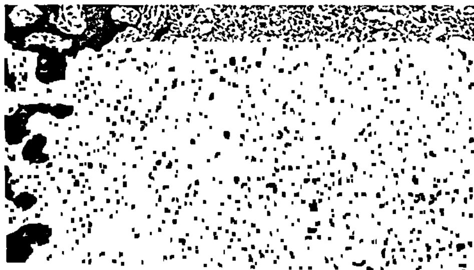


Fig 11 Osteoclastic osteosarcoma lumbar spine male mouse 182 days after combined oestrogen and ^{90}Sr treatment Van Gieson × 140



Fig 12 Sternal vertebrae 67 days after first oestrogen and 60 days after ^{90}Sr injection Marrow cavity occupied by newly formed bone Van Gieson × 140



Fig 9

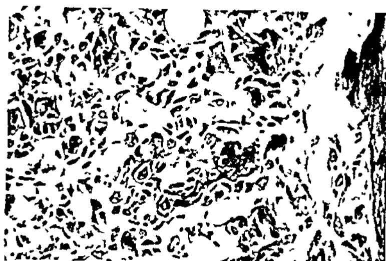


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Table 7 *(cont)*

Oestrogen + ^{90}Sr					
60	83	113	143	173	203
956 ± 120	1208 + 142	968 ± 54	1280 ± 124	1544 ± 245	1800 ± 305
—	—	13 48 ± 0 18	12 9 ± 0 30	12 36 ± 1 01	12 24 ± 0.22
7 90 + 0 35	7 48 ± 0 19	7 62 ± 0 27	7 39 ± 0 15	6 62 ± 0 56	6 81 ± 0 29
142 28 + 13 40	112 1 + 2 08	116 5 ± 7 87	101 56 ± 11 10	96 56 ± 19 4	110 46 ± 8 48
7 64 ± 1 39	5 66 ± 0 75	9 48 ± 3 09	18 36 ± 7 10*	6 56 ± 1 71	13 60 + 2 19
—	206 800 ± 9 970	151 000 ± 25 300	265 600 ± 18 950	348 400 ± 32 200	362 600 ± 27 300

Discussion

The combination of ^{90}Sr and oestrogenic hormones has revealed a highly potentiated effect in mice as compared with either treatment alone A highly significant reduction of the mean survival time was thus noted Despite this the bone tumour rate was significantly enhanced The tumour induction time was also significantly shortened and the histologic appearance of the tumour modified

As evidenced by histologic observation elsewhere (NILSSON 1962, 1970) it has been found that ^{90}Sr brings about a severe suppression of the osteoblastic cell population This depletion is later followed by an increased cellular activity and proliferations at circumscribed areas along the endosteal linings or more diffusely in the bone marrow cavity The start of this proliferation is dependent upon dose, but it usually commences within 5 to 6 months after optimal doses of ^{90}Sr These cells are, however, functionally defective and morphologically atypical, fusiform elements which may undergo neoplasia Oestrogenic hormones induce an intense formation of new bone When combined with ^{90}Sr, this bone apposition is, however, largely inhibited (Fig 7) The osteoblasts are replaced by functionally defective fusiform cells producing an abundance of fibrous tissue instead of bone Numerous osteoclasts attacking the 'oestrogenic' bone also appear These histologic events appear very early, are more intense and more widespread than after ^{90}Sr alone The reason for this may be related to the stimulating effect of oestrogen on the cell compartment, as shown by SIMMONS (1962) He states that the primary effect of these hormones on the skeleton is to stimulate the onset of the modulations of the undifferentiated marrow cells to form osteoblasts Such osteogenic potentials of reticular cells leading to a rapid transformation into

Table 7

Blood data and weights of spleen and thymus of mice treated with ^{90}Sr and oestrogen + ^{90}Sr

Day after ^{90}Sr	Mice, serially killed			
	^{90}Sr			
	60	113	173	203
Leukocytes	1972 ± 264	2156 ± 162	2212 ± 171	2384 ± 186
Haemoglobin (g/100 ml blood)	—	14 02 ± 0 97	12 64 ± 0 55	12 88 ± 0 32
Erythrocytes 10^6	8 59 ± 0 21	7 64 ± 0 22	6 55 ± 0 30	6 55 ± 0 40
Weight of spleen mg	102 14 ± 3 02	90 06 ± 6 71	62 00 ± 2 67	72 70 ± 1 47
Weight of thymus mg	31 88 ± 1 46	21 32 ± 2 26	11 60 ± 2 47	14 56 ± 0 50
Thrombocytes	—	300 000 ± 25 700	298 000 ± 31 100	477 000 ± 84 900

* Two cases of unilateral lymphomas

alone there appeared to be a slightly diminished cellularity of the bone marrow as compared to control animals

There was also a general tendency to bleeding in the mice in experiment II Out of the 20 mice dying spontaneously 3 died of haemothorax and haemocoelia Small bleedings were also observed in all mice killed on days 60 and 83 and in 4 out of 5 on day 113 Thrombocyte counts were only slightly depressed and may not be related to the haemorrhages observed (Table 7) The weight of the thymus among oestrogen + ^{90}Sr treated mice was significantly diminished and that of the spleen increased ($0\ 02 > p > 0\ 01$) up to day 113 as compared with mice given only ^{90}Sr (Table 7) The increased weight of the spleen was due largely to an increased extramedullary haematopoiesis of all types of cells The number of leucocytes in the oestrogen + ^{90}Sr group was much more reduced than in the ^{90}Sr group, up to day 113 the difference was significant ($p < 0\ 001$) (Table 7) In the group given oestrogen + 0 4 μCi ^{90}Sr/g the weight of the spleen increased to 5 to 10 times normal weight In many of these cases an enormous proliferation of myeloblastic elements and granulocytes took place in a

poiesis was successively reduced

In experiment II two cases of unilateral lymphoma were detected among oestrogen + ^{90}Sr treated mice

reduced In combination with oestrogenic hormones this effect (Table 7) is even more severe This deficiency and the fact that oestrogenic hormones exert a strong suppression on the immunologic responsiveness (THOMPSON & RUSSE 1965, THOMPSON et coll 1966) might suggest the possibility of peracute infection without visible pathologic lesion A general immunologic suppression, although not tested, might also have occurred in this investigation as judged from the severe damage to the thymus, spleen and bone marrow

The reason for the variation of the leukaemia incidence between the different groups (Table 3) cannot be satisfactorily explained and will be further investigated, as well as the relation between the treatment and frequency of thymic and non thymic lymphomas

Acknowledgement

This investigation was carried out as part of the programme of the European Late Effects Project Group (EULEP)

SUMMARY

Groups of male and female CBA mice were treated with oestrogenic hormone alone, ^{90}Sr alone or with oestrogen +^{90} Sr Among the males given both oestrogen and ^{90}Sr there was a mean number of 4 2 bone tumours/mouse with a mean induction time of 194 1±2 6 days as compared with 2.3 among those given only ^{90}Sr The mean induction time for the latter was 316 1±1 1 days The female mice treated with ^{90}Sr+oestrogen developed 4 1 bone tumours/mouse and those *given ^{90}Sr alone 2 0* The induction times were 251 0±4 1 and 379.3±9 7 days respectively No bone tumours were found in the group of mice treated with only oestrogen Osteoclastic and mixed osteosarcomas were frequent in combination treatment groups in contrast to the result when ^{90}Sr was given alone

ZUSAMMENFASSUNG

Gruppen
alleine,
Östroger
Knocher … aus mit einer mittleren Induktionszeit von 194 1±2 6 Tagen verglichen mit 2,3 bei denen die nur ^{9} Sr erhalten hatten Die mittlere Induktionszeit fur letztere betrug 316 1±1 1 Tage Die mit ^{90}Sr+Östrogen behandelten Weibchen entwickelten 4,1 Knochentumoren/Maus und die nur mit ^{90}Sr behandelten Weibchen 2 0 Die Induktionszeiten betrugen 251 0±4 1 bzw 379,3+9,7 Tage Bei der Gruppe von Mausen, die nur mit Östrogen behandelt worden war fanden sich keine Knochentumoren Osteoklastische und gemischte Osteosarcome waren bei den kombiniert behandelten Gruppen im Gegensatz zu den nur mit ^{90}Sr behandelten Gruppen haufig

RÉSUMÉ

Des groupes de souris CBA mâles et femelles ont ete traités par l…
seule par le ^{90}Sr …
les oestrogenes
avec un temps …

preosteoblasts and osteoblasts come into effect, however, only when the cells are approached by bony surfaces growing out from the endosteum This means that ^{90}Sr irradiates not only a much more numerous cell population, but also a very alert, constantly stimulated cell population Both these factors might be of importance for the increased tumour frequency found The tumour frequency, however, was the same whether ^{90}Sr was given during or after the last oestrogen injection This seems to indicate that the overpopulation is a most decisive factor, since the greater the population irradiated, the greater the chances for malignant clones to develop Intimately associated with this enhanced development and earlier appearance of malignant clones, micro- and macroscopic tumours are probably also immunological factors This is indicated by a threefold prolonged skin allograft survival and an impaired primary haemaglutination response when oestrogens are combined with external irradiation (THOMPSON et coll 1966), and by the fact that ^{90}Sr-induced bone tumours are antigenic (NILSSON et coll 1972).

The occurrence of osteoclastic and mixed osteosarcomas, which are extremely rare when ^{90}Sr alone is given, might be related to the fact that osteoclasts were numerous already in early stages preceding tumour development

The relation of irradiation to the increased tumour frequency is not clear From Table 3 and Fig 2 it is seen that the tumour incidence in group E (female mice which were given oestrogen and 0 4 μCi ^{90}Sr/g body weight) was almost a factor of two (1 8) greater than in group B (male mice given only ^{90}Sr, 0 8 μCi/g body weight) Also the tumour induction time in the former group was significantly shorter ($p < 0\ 001$) With reservations for possible sex differences in the rate of tumours these facts seem to indicate that irradiation does not play a decisive role in the enhanced occurrence of tumours in the groups with combined treatment This relation is, however, studied in a separate investigation and will be discussed elsewhere

In many cases pathology was practically negative As THOMPSON et coll (1965) have stated, earlier anaemia seems to be of little importance for the early mortality Despite the conspicuous destruction of the marrow found in this investigation, the spleen seems to be able to compensate for the erythropoietic insufficiency of the marrow A factor of consequence for the enhanced early mortality might, however, as previously suggested by THOMPSON et coll (1965), be related to the strong impairment of the myeloid and lymphatic tissues (Table 7), which makes them unable to achieve levels necessary to maintain survival This is particularly true since ^{90}Sr doses of the same size as used in this investigation have been shown to induce a maximum depression of leucocyte counts around 16 days after the injection of the nuclide (NILSSON 1962) At this time the neutrophils are almost eliminated and the lymphocytes strongly

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CARCINOID-ISLET CELL TUMORS

S L GAMMILL, R. WEICHERT, S L SMITH, R FONT and R. INGRAFFIA

Pathologists have long experienced difficulties in distinguishing between 'carcinoid' and 'islet cell' tumors histologically Many medical publications have shown a striking overlap in the secretions of these tumors One of the co-authors of this paper (R W) described a series of patients with these tumors (26, 27, 28) in which the overlap in function among them was convincing enough to hypothesize that they are actually variations of the same tumor, should be grouped together as such, and should be referred to as carcinoid islet cell tumors The original reports concerned 21 patients We have added 7 patients to that number and have performed angiography on 6 of these and on 2 patients from the previous series We shall describe and discuss these findings and how they may be used in the diagnosis and outline of the extensiveness of these tumors Also, we shall use this information to lend further support to the premise that these are actually variations of the same tumor and should be referred to collectively as carcinoid islet cell tumors

Submitted for publication 31 July 1972

celles qui avaient reçu seulement le ^{90}Sr Le temps moyen d induction pour ce dernier groupe etait de 316,1 ± 1,1 jours Les souris femelles traitees par ^{90}Sr + oestrogene ont eu 4 1 tumeurs osseuses par souris et celles qui n avaient reçu que ^{90}Sr ont eu 2 0 tumeurs par souris Les temps d induction étaient respectivement de 251 0 ± 4 1 et 379 3 ± 9 7 jours Les souris traitées seulement par l oestrogène n ont pas presente de tumeur osseuse Les ostéo sarcomes osteoclastiques et les osteosarcomes mixtes ont ete *fréquents dans les groupes traites* par l association oestrogene + ^{90}Sr, a la difference des resultats donnes par le ^{90}Sr seul

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formation in oestrogen treated



Fig 2 Celiac angiography Large superior pancreatoduodenal artery (⟵→), tumor vascularization in head of pancreas (→)

torrhea persisted, weight loss, recurrence of abdominal pain BP 192/130 Triolean absorption test small bowel biopsy VMA and regitine tests normal Aortography absent right renal artery Celiac angiography accumulation of contrast medium in distribution of gastroduodenal artery and enlarged pancreatoduodenal artery (Fig 2) Superior mesenteric angiography normal Course Deteriorated slowly One day before death, symtoms of hypoglycemia blood sugar 60 responded favorably to glucose intravenously Autopsy Tumor in head of pancreas with necrotic mestases to liver Cystic right kidney *Summary* Duodenal ulcer diarrhea, steatorrhea suggesting that this tumor secreted gastrin and produced a variation of the Zollinger Ellison syndrome Pathologic diagnosis malignant islet cell tumor Our analysis Carcinoid islet cell tumor of pancreas that secreted gastrin and probably insulin It is possible that the episode of hypoglycemia was caused by depleted glycogen [illegible]

[illegible] duodenal ulcer tumor in wall of duodenum that had been cut through and part of it left in the duodenum Readmission five months later for consideration of re-operation. She had been asymptomatic in the interval 5HIAA, blood sugar normal, no operation 1971 No symptoms for seven years Celiac angiography attenuation of gastroduodenal artery and faint accumulation of contrast medium in distribution of this artery that disappeared on later films uncertain whether the accumulation was in the tumor that had been left or in the wall of the duodenum Superior mesenteric angiography normal *Summary* Classical Zollinger Ellison syndrome in a patient with a duodenal tumor Pathologic diagnosis carcinoid tumor Our analysis Carcinoid islet cell tumor of duodenum that secreted gastrin and produced the classical Zollinger Ellison syndrome

Case 4 White woman, aged 53 1963 Easily fatigued, postprandial flushing of head and neck for six months Positive 5HIAA on three separate occasions Upper gastrointestinal



(Fig. 1 a) Celiac angiography, arterial phase. Large pancreatoduodenal artery with a hypervascularized tumor in its distribution (→). b) Capillary phase. Accumulation of contrast medium (→) persists and is drained by a large abnormal early filling vein crossing the midline (↔).

Case reports

Case 1. Male Negro aged 67. 1954: Upper gastrointestinal bleeding. Radiography: duodenal ulcer. Gastric laboratory analysis: normal. Blood sugar 66 (normal 80—120). Op.: 80% gastrectomy. Pathologic examination: 4 duodenal bulb ulcers and a duodenal carcinoid tumor that had been cut through and part of the tumor left in the patient. 1955: Two marginal ulcers, one in the stomach and one in the small bowel. Op.: total gastrectomy. Following surgery, no more peptic ulcer symptoms. Blood sugars 62, 73. 1956: Nephrolithiasis requiring heminephrectomy. Blood sugar 75, elevated serum calcium, lowered serum phosphorus. 1961: Blood sugar 55, elevated serum calcium, lowered serum phosphorus. 1970: Celiac angiography (Fig. 1): accumulation of contrast medium with large early filling draining vein in distribution of gastroduodenal artery, probably in the duodenal tumor that had been left in the patient. Superior mesenteric angiography normal. 5HIAA (normal). Serum serotonin and gastrin lost in the laboratory. *Summary*: Severe peptic ulcers nonresponsive to partial gastrectomy but responsive to total gastrectomy; multiple decreased blood sugar levels suggesting hyperinsulinism; multiple increased calcium and decreased phosphorus values suggesting a parathyroid adenoma. Pathologic diagnosis: carcinoid tumor of duodenum. Our analysis: Multiple endocrine adenomatoses with a parathyroid adenoma and a carcinoid islet cell tumor that secreted gastrin and insulin. It is possible that the carcinoid islet cell tumor secreted parahormone but probably he had more than one tumor.

Case 2. Male college student aged 21. 1965: Right upper quadrant abdominal pain and jaundice. Medical management with fair result. 1967: Persistent abdominal pain and jaundice, diarrhea, followed in two months by steatorrhea. BP 134/96. Radiography: duodenal ulcer. Serum amylase 698. Gastric analysis not done. Liver biopsy: minimal inflammation. Laparotomy: common bile duct stricture, small firm nodule in head of pancreas, cystic right kidney. Choledochoduodenostomy. Following operation disappearance of pain. 1968: Stea-

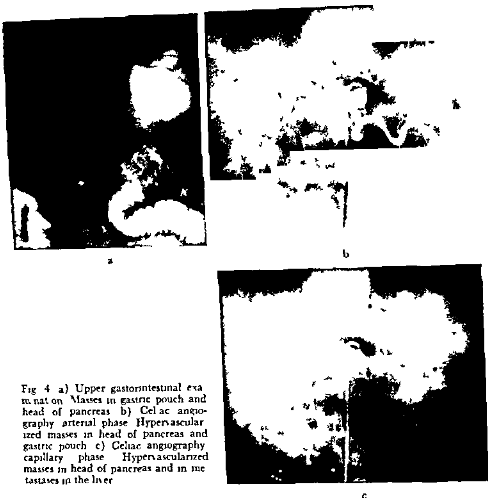


Fig 4 a) Upper gastorintestinal examination Masses in gastric pouch and head of pancreas b) Celiac angiography arterial phase Hypervascularized masses in head of pancreas and gastric pouch c) Celiac angiography capillary phase Hypervascularized masses in head of pancreas and in metastases in the liver

gastrointestinal tract and lung tumor either primary or metastatic, that secreted serotonin and gastrin or another ulcerogenic substance

Case 6 White woman aged 67 1969 Dizziness, disorientation and shock relieved by intravenous glucose Blood sugar 50 during one of these attacks Tolbutamine tolerance test positive for hyperinsulinism Celiac angiography arterial phase, hypervascularity of body and tail of pancreas encasement of splenic artery Capillary phase puddling body and tail of pancreas multiple liver metastases Upper gastrointestinal series normal Op laparotomy, tumor of pancreas unresectable Pathologic diagnosis Islet cell tumor Our analysis Carcinoid islet cell tumor of pancreas metastatic to the liver that secreted insulin 1971 Patient re admitted to hospital with unexplainable vomiting and diarrhea We considered that the

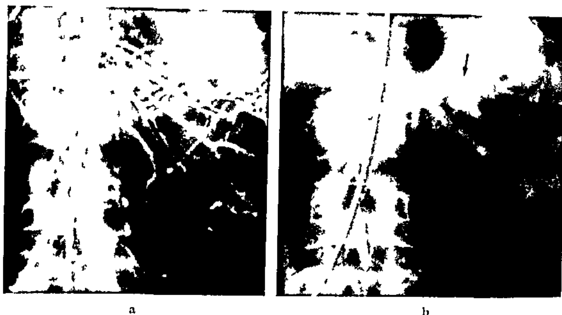


Fig 3 a) Superior mesenteric angiography arterial phase Tumor vascularization in jejunum (→→) b) Capillary phase Accumulation of contrast medium (→→) persists large abnormal early filling draining veins crossing midline (→)

series and blood sugar were normal Superior mesenteric angiography (Fig 3 a) accumulation of contrast medium in jejunum in the arterial phase Large early filling draining veins from tumor in the capillary phase (Fig 3 b) Celiac angiography metastases in liver Patient flushed during injections Op resection of jejunal tumor Pathologic diagnosis carcinoid tumor Our analysis Carcinoid islet cell tumor of jejunum metastatic to the liver that secreted serotonin

Case 5 Male Negro aged 66 1942 Flushing tachycardia headaches nausea weakness A diagnosis of peptic ulcer resulted in his discharge from the Army 1946 Perforated prepyloric ulcer closed by Graham closure technique Gastric analysis normal 1948 Persistent abdominal pain Op subtotal gastrectomy Pathology prepyloric ulcer and tumor incidentally found in wall of duodenum 1954 Slow gastrointestinal bleeding that had persisted for several years Op part of duodenal tumor removed from gastric pouch 1957 Solitary nodule in lung 5HIAA positive Op lobectomy of lung removal of more tumor from gastric pouch 1959 5HIAA pos 1963 5HIAA neg 1968 Slow gastrointestinal bleeding continued Radiography masses in gastric pouch and head of pancreas (Fig 4 a) Celiac angiography accumulation of contrast medium in wall of gastric pouch and head of pancreas (Fig 4 b) and in liver on later phase films *Summary* Severe peptic ulcer disease with clinical and laboratory evidence of serotonin secretion *Comments* It seems reasonable to assume that either this tumor originated in the pancreas and spread to the duodenum and stomach and metastasized to the lung or that the patient had multiple tumors It is doubtful whether the tumor metastasized to the head of the pancreas The growth apparently secreted serotonin until 1963 but not later Pathologic diagnosis carcinoid tumor in lung and gastrointestinal tract Our analysis Carcinoid islet cell tumors primary in pancreas and of

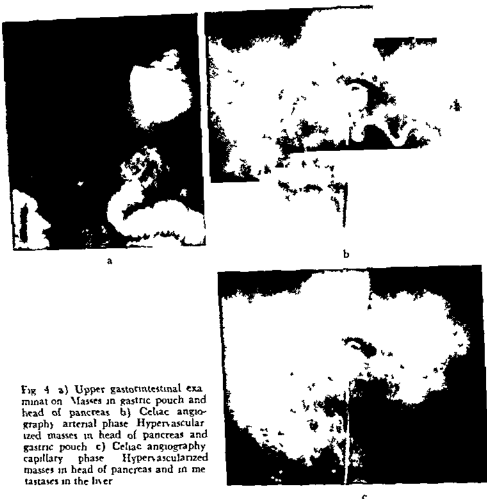


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a

b

Fig. 5 a) Superior mesenteric angiography, arterial phase. Hepatic artery originates from superior mesenteric artery. b) Superior mesenteric angiography, capillary phase. Hypervascularization in head and body of pancreas and in metastases in liver.

Table

Potential of 28 carcinoid islet cell tumors to secrete more than one hormone (I) as well as only one (II) or none (III). Twenty-one of these tumors have been previously published by R. W. (27, 28, 29) from the clinical standpoint. No angiographic results from these patients have been published

I 16 ulcerogenic tumors
- 5 Zollinger Ellison syndrome
- 3 Zollinger Ellison syndrome multiple endocrine adenomatoses
- 1 Zollinger Ellison syndrome, insulin
- 3 Peptic ulcer
- 1 Peptic ulcer, multiple endocrine adenomatoses
- 1 Peptic ulcer, insulin, multiple endocrine adenomatoses
- 2 Peptic ulcer serotonin

II 2 non ulcerogenic tumors secretory
- 1 serotonin
- 1 insulin

III 10 non secretory

tumor was secreting gastrin at that time but were unable to obtain a serum gastrin level or gastrointestinal series

Case 7 White male, aged 65 1969 Abdominal mass discovered during mass health examination Upper gastrointestinal series normal Superior mesenteric angiography accumulation of contrast medium in pancreas with multiple hypervascularized metastases to liver (Fig 5) Op resection of most of tumor, followed by radiation therapy Pathologic diagnosis " argentaffin type neoplasm" Our analysis Non secreting carcinoid islet cell tumor of pancreas

Discussion

We have reviewed a total of 28 patients with carcinoid-islet cell tumors, the clinical findings of which are summarized in the Table The peptic ulcers mentioned were severe peptic ulcers, but we chose to class them as such rather than as Zollinger-Ellison syndromes, since these patients did not have the classical Zollinger-Ellison features Since published evidence clearly shows that gastrin secreting tumors may produce variations of the Zollinger-Ellison syndrome consisting of diarrhea, steatorrhea, simple peptic ulceration or marginal ulceration, and since 20 per cent of patients with gastrin secreting tumors may not have hyperacidity, we could have as easily classified most of these patients as Zollinger-Ellison syndrome (27) The 28 carcinoid islet cell tumors were located as follows 22 in the duodenum or small bowel, 4 in the pancreas and 2 in the pancreas and duodenum Thirteen of the 16 ulcerogenic tumors were in the duodenum

or small bowel, 1 in the pancreas and 2 in the pancreas and duodenum Of the 3 serotonin secreting tumors, 2 were located in the small bowel and 1 in the pancreas and duodenum *It is evident that tumors of the pancreas and duodenum overlap significantly in function*

From the Table, it is evident that carcinoid islet cell tumors may secrete nothing gastrin or insulin or serotonin or combinations of these hormones True we had to assume the secretory functions of some of these tumors from clinical findings since serum gastrin and serum insulin level determinations have only recently become readily available, but the clinical evidence seems reasonably conclusive For instance, if a patient had the classical Zollinger Ellison syndrome as case 3 did it seems reasonable to assume that the tumor secreted gastrin If patients had severe peptic ulcers, or other findings that have been intimately linked to gastrin secreting tumors (27), as in cases 1, 2 and 5 it again seems reasonable to assume that these tumors secreted gastrin or at least some ulcerogenic substance *This assumption is further supported by a large autopsy series* in which there was a 38 per cent incidence of peptic ulceration associated with carcinoid tumors as opposed to a 5 5 per cent incidence in patients without 'carcinoid tumors (15) It is to be doubted whether histamines or serotonin are the culprits in ulcer production in these patients as ZOLLINGER et coll have shown with fair certainty through laboratory experiments that gastrin will produce the Zollinger Ellison syndrome whereas histamine and extracts of carcinoid tumors will not We suppose another ulcerogenic substance could be secreted by these tumors but this remains to be proven

Tumors secreting serotonin may either be found in the duodenum small bowel or pancreas if we assume that the tumor in the pancreas in case 5 had a part in the secretion of the serotonin

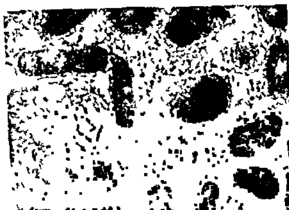
Nine of the patients with duodenal tumors had the classical Zollinger Ellison syndrome This strongly suggests that these tumors secreted gastrin and further confirms the overlap in function between pancreatic and duodenal carcinoid islet cell tumors This is a striking observation since most of the gastrin secreting tumors producing the Zollinger Ellison syndrome have previously been found *within the pancreas*

In earlier classifications of carcinoid tumors they were divided into foregut (lung stomach and duodenum), midgut (pancreas small bowel) and hindgut (appendix colon) tumors The foregut and midgut tumors were often secretory but the hindgut tumors were usually not There were 10 foregut or midgut tumors *in this series that apparently secreted no hormones* Perhaps their development was similar to the hindgut tumors We have no other explanation as to why they were non secretory

One patient, previously reported in another publication (29) who had a



a



b

Fig 6 a) Electron micrograph (multiplied × 31 500) of cytoplasmic granules that have been associated with gastrin secretion and have been found in normal islet cells These granules were in a duodenal tumor b) Electron micrograph (multiplied × 31 500) of cytoplasmic granules from an ileal tumor found in same patient as in (a) The granules with halos have been associated with the secretion of serotonin (Reprinted with the permission of Cancer and J B Lippincott Co and previously published in that journal (29))

duodenal ulcer non responsive to medical management over a 10-year period, developed hypersecretions and an acute outlet obstruction of the stomach No gastric analysis was performed He was operated on and had a severely scarred duodenal bulb that had produced a total obstruction at the pylorus A duodenal tumor and eight ileal tumors were found and resected An electron microscopic examination of the duodenal tumor showed cytoplasmic granules, the so-called delta granules that have been found in normal islet cells and have also been associated with the secretion of gastrin Granules from an ileal tumor in the same patient contained cytoplasmic granules that have been associated with the secretion of serotonin (29) (Fig 6)

The angiographic findings associated with 'islet cell' tumors and 'insulinomas'

that have been presented in the literature are quite similar to the findings present in the tumors presented in this paper (3, 8, 10, 14, 16, 18, 24) Only two reports could be found that described angiographic findings associated with 'carcinoid tumors (REUTER et coll, ROSCH & STECKEL) The tumors presented in the present report had much more prominent vascularity than the ones reported by REUTER et coll and were strikingly similar to the findings associated with 'islet cell' tumors We conclude from these observations that these tumors cannot be separated angiographically When metastases from these tumors are present in the liver, they are readily demonstrable as they produce an intense accumulation of contrast medium Necrotic metastases which no longer have a blood supply form an exception

Since the pathologist cannot separate 'carcinoid' and 'islet-cell' tumors histologically, these tumors cannot be separated as to function because 'carcinoid tumors may secrete gastrin or insulin in addition to serotonin or histamine (12, 21, 26, 27) and 'islet-cell' tumors may secrete serotonin, insulin or both (1, 2, 4, 6, 7, 9, 11, 13, 17, 25, 26), and since the angiographic findings are the same, it appears that there is no basis upon which to separate them These two growths should therefore be grouped together, thought of as variations of the same tumor, and referred to collectively as carcinoid-islet cell tumors

Conclusion

(1) According to histologic, pathologic, functional and angiographic findings, 'carcinoid' and 'islet cell' tumors are actually variations of the same tumor and should be referred to collectively by one term carcinoid islet cell tumors

(2) These tumors are capable of secreting more than one hormone and a patient with a carcinoid islet cell tumor may have other neuroendocrine tumors (multiple endocrine adenomatoses) and should therefore have a complete clinical and laboratory endocrine analysis (5HIAA, serum gastrin, *tolbutamide tolerance* test, serum insulin levels, serum calcium, and serum phosphorus, etc)

(3) Patients with recalcitrant peptic ulcers, marginal ulcers, unexplained diarrhea or steatorrhea may have a carcinoid islet cell tumor that secretes gastrin or another ulcerogenic hormone and should therefore be considered for an endocrine analysis and *visceral angiography*

SUMMARY

The difficulties in distinguishing between carcinoid and islet cell tumors are discussed *According to* histologic pathologic functional and angiographic findings these tumors are actually variations of the same tumor and should be referred to as carcinoid islet cell tumors

ZUSAMMENFASSUNG

Die Schwierigkeiten, zwischen einem Karzinoid und einem Inselzell Tumor zu unter scheiden werden diskutiert Entsprechend den histologischen, pathologischen funktionellen und angiographischen Befunden sind diese Tumoren eigentlich Varianten ein und desselben Tumors und sollten als Karzinoid Inselzell Tumoren bezeichnet werden

RÉSUMÉ

Les auteurs examinent les difficultes qu il y a pour distinguer les tumeurs carcinoides des tumeurs a cellules insulaires D apres les resultats histologiques, fonctionnels et angiographi ques ces tumeurs sont en realite des variantes de la même tumeur et devraient etre appelees tumeurs carcinoides a cellules insulaires

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ANTITUMOUR EFFECT OF LASER RADIATION

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The optical quantum generators (lasers) are physical devices that produce the electromagnetic radiation whose wavelength lies within the range of infrared to ultraviolet regions of the spectrum The outstanding physical properties of laser emission—the high coherence, monochromaticity, strict directivity and, especially, tremendous energy density—were the main motives that gave impetus to the present research on the antitumour properties of the laser beam

Numerous experimental investigations conducted on the cultures of malignant tumour cells (Rounds et coll 1965 a, b, Kavetsky & Gamaleya 1967, 1968, Gamaleya & Pasetchnik 1969) as well as on a number of transplanted and induced tumours (McGuff et coll 1963, McGuff 1966 a, Fine et coll 1964 a, Klein et coll 1965, Minton 1965 a, b, Goldman 1966 a, Kavetsky et coll 1968 1969 a, b, Kozlov et coll 1972) convincingly demonstrated that laser beams can destroy malignant neoplasms These experimental data suggested that the effect of laser radiation depends upon the biologic peculiarities of malignant neoplasms as well as on the type and physical parameters of the laser applied

The influence of the pulse energy and the irradiation rhythm on the growth of experimental tumours which differed from one another in certain biologic properties, were consequently investigated An attempt was made to enhance the

Submitted for publication 6 September 1972

antitumour effect of laser radiation by means of such ionizing radiation as fast electrons

Materials and Methods

The experiments were conducted in male, white nonbred mice as well as in mice of CC_5-Br and C_{57}Bl lines The nonpigmented malignant tumours such as the *Ehrlich tumour, forestomach planocellular carcinoma* or melanin containing malignant neoplasms, such as the Harding-Passy and B_{16} melanomas, were *transplanted under the skin of the hind right extremity* of the animals Tumours, which had reached 5 to 8 mm (series I—VI, X, XII) *or 9 to 12 mm* (series VII, VIII, IX, XI) in diameter, were irradiated by laser beams in a single or during many seances When the multiseance method was used, the tumours were exposed daily during the test to 1 to 2 pulses, with the one-seance method the whole surface of the tumour was irradiated each time by *3 to 7 pulses* The source of irradiation was a laser installation, type ГОС-1000, which is a solid state laser in which glass activated by triple valent neodymium is used as working substance The pulsed laser generates electromagnetic radiation of 10 000 Å wavelength The maximum radiation pulse energy attains 1 000 J with a pulse duration of 10^{-3} s Radiation with an output energy of 300 to 350 and 700 to *750* J was *focussed* during the tests by means of a convexo-convex lens with a *focal* distance of 50 cm on to the tumour surface to a light spot area of 2 5 to 5 0 *mm*^{2}, the energy density of the laser *radiation amounted* to 15 000 to 25 000 J/cm^2 The total dose of laser radiation incident upon the tumour equalled 600—1 700—2 100—6 000 J The hair of the animals over the tumours was depilated mechanically before the application

The influence of the laser radiation applied in combination with fast electrons on the growth of the tumours was investigated in series IX—XII The animals in each of the series in these experiments were divided into four groups Along with the control mice were groups of animals whose tumours were exposed either to the irradiation by laser beams only, to the irradiation of fast electrons or to the combined treatment by *both* these irradiations *The irradiation of tumours* by laser beams in these series of experiments was performed by 3 to 8 pulses in one *seance with a beam output* energy *of 700 to 750* J

The fast electrons were generated by a linear electron accelerator, type ЛУЭ 25 The tumours were irradiated by a scanning beam of electrons with an energy of 22 MeV, at a dose power of 90 to 100 rad/min three times a week over two weeks The single dose of electron irradiation in all series amounted to 1 000 rad with a cumulative dose of 6 000 rad For the purpose of irradiation a ten section radially symmetric bench of plexiglass with the lower wall 1 cm thick was used

Table 1

Effect of neodymium laser on the growth of implanted tumours

Series	Tumour	No. of animals: Control	No. of animals: Test	Quantity of irradiation seances	No. of pulses in a seance	Pulse energy (J)	Cumulative dose	Weight of tumour (g): Control	Weight of tumour (g): Test	P	Inhibition (%)
I	*Ehrlich* tumour	10	9	3	1–2	300–350	900–1 800	8.4±0.6	8.2±0.4	> 0.1	2
II	Ehrlich tumour	10	10	2	1–3	300–350	600–1 700	7.1±0.5	6.9±0.6	> 0.1	2
III	Ehrlich tumour	10	10	5	1	700–750	3 500–3 700	6.6±0.4	5.1±0.3	< 0.05	22
IV	Ehrlich tumour	10	10	3	1–2	700–750	2 500–3 800	6.7±0.4	5.0±0.6	< 0.05	25
V	Fore stomach plano-cellular carcinoma	13	11	4	1–2	700–750	2 500–4 500	5.4±0.6	1.6±0.3	< 0.001	69
VI	Harding Passy melanoma	11	10	5	1–2	700–750	3 000–5 000	7.0±0.2	2.6±0.2	< 0.001	63

The mice with the tumours transplanted into the hind limb were tied to the centre of the bench by the paws so that the tumours were closely adjacent to one another. This method of irradiation enabled disposition of the tumours within the zone of 90 per cent isodose. Paraffin wax 3 cm thick was placed under the frame to improve the distribution of the dose along the irradiated volume. Each seance of irradiation by electrons was controlled by an ionizing chamber (monitor) of these dosimeter 'automatic-dose', the latter was graduated by means of ferrosulphate dosimetry to eliminate the errors while determining the absolute absorbed doses. The dose measuring total error did not exceed ten per cent which,

antitumour effect of laser radiation by means of such ionizing radiation as fast electrons

Materials and Methods

The experiments were conducted in male, white nonbred mice as well as in mice of $CC_{57}Br$ and $C_{57}Bl$ lines The nonpigmented malignant tumours such as the Ehrlich tumour, forestomach planocellular carcinoma or melanin containing malignant neoplasms, such as the Harding-Passy and B_{16} melanomas were transplanted under the skin of the hind right extremity of the animals Tumours, which had reached 5 to 8 mm (series I—VI, X, XII) or 9 to 12 mm (series VII, VIII, IX, XI) in diameter, were irradiated by laser beams in a single or during many seances When the multiseance method was used, the tumours were exposed daily during the test to 1 to 2 pulses, with the one-seance method the whole surface of the tumour was irradiated each time by 3 to 7 pulses The source of irradiation was a laser installation, type ГОС-1000, which is a solid state laser in which glass activated by triple valent neodymium is used as working substance The pulsed laser generates electromagnetic radiation of 10 000 Å wavelength The maximum radiation pulse energy attains 1 000 j with a pulse duration of 10^{-3} s Radiation with an output energy of 300 to 350 and 700 to 750 j was focussed during the tests by means of a convexo-convex lens with a focal distance of 50 cm on to the tumour surface to a light spot area of 2 5 to 5 0 mm², the energy density of the laser radiation amounted to 15 000 to 25 000 j/cm² The total dose of laser radiation incident upon the tumour equalled 600—1 700—2 100—6 000 j The hair of the animals over the tumours was depilated mechanically before the application

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The fast electrons were generated by a linear electron accelerator, type ЛУЭ 25 The tumours were irradiated by a scanning beam of electrons with an energy of 22 MeV, at a dose power of 90 to 100 rad/min three times a week over two weeks The single dose of electron irradiation in all series amounted to 1 000 rad with a cumulative dose of 6 000 rad For the purpose of irradiation a ten section radially symmetric bench of plexiglass with the lower wall 1 cm thick was used

Table 3

Influence of laser radiation and high speed electrons on the growth of melanomas

Series	Tumour	Groups	No. of animals n	Size of tumours (mm)	No. of pulses	Cumulative dose (J)	Weight of tumour (g)	p	Inhibition (%)
IX	Harding Passy melanoma	Control	9				11.4±0.7		
		Laser	9	10–12	5–8	3 500 6 000	3.6±0.6	<0.001	68
		Laser+ electrons	10				0.8±0.1	<0.001*	92
		Electrons	9				4.1±0.9	<0.001	64
X		Control	10				9.0±0.4		
		Laser	9	5–6	3–5	2 100 3 000	2.4±0.3	<0.002	73
		Laser+ electrons	9				0.9±0.2	<0.001*	90
		Electrons	9				4.0±0.5	>0.02	55
XI	B_{16} melanoma	Control	9				5.9±0.6		
		Laser	9	10–12	4–7	2 800–5 600	2.4±0.3	<0.001	59
		Laser+ electrons	9				0.3±0.1	<0.001 *	95
		Electrons	10				2.3±0.3	<0.01	60
XII		Control	9				4.7±0.7		
		Laser	9	5–6	3–5	2 100–3 700	1.5±0.4	<0.001	68
		Laser+ electrons	9				0.4		91
		Electrons	10				1.9±0.3	<0.001	59

* The probability value (p) given for the difference with the laser group

Results

The investigation of the pulse energy influence on the antitumour effect of the laser beams was performed in series I—IV on the Ehrlich tumour, this having reached an average diameter of 5 to 8 mm was irradiated during the test by 1 to 2 pulses of the focussed laser with output energies of 300 to 350 and 700 to 750 J every other day. A certain antitumour effect was obtained only in the case of the tumour being exposed to the laser beams with an energy of 700 to 750 J. When the Ehrlich tumour was exposed to laser radiation of 300 to 350 J energy its growth did not substantially differ from the growth in the control group (Table 1).

Table 2

Influence of irradiation rhythm on the antitumour effect of neudymium laser

Series	Tumour	Groups	No of animals	No of radiation seances	No of pulses in a seance	Pulse energy (J)	Cumulative dose (J)	Weight of tumour (g)	p	Inhibition (%)
VII	Fore stomach plano-cellular carcinoma	Control	9					5 7±0 4		
		Multi-seance irradiation	10	4	1—2	700—750	3 000—6 000	3 0±0 2	< 0 001	47
		Single seance irradiation	10	1	3—7	700—750	2 100—5 000	2 1±0 3	< 0 001	63
VIII	Harding Passy *mela*noma	Control	10					8 4±0 9		
		Multi-seance *irra*diation	9	4	1—2	700—750	3 000—6 000	4 8±0 4	< 0 001	43
		Single seance irradiation	10	1	4—7	700—750	3 000—5 000	2 0±0 6	< 0 001	76

according to the recommendations of the International Commission on Radiation Units and Measurements (ICRU, Report 10, 1962) corresponds to the conditions of uniform irradiation (Class A)

The histologic test of Harding-Passy *melanoma was performed* one hour after a single irradiation by focussed laser with an energy of 750 J The test sections were prepared in the plane parallel to the beam, the histologic preparations were coloured with haematoxylin-eosin The antiblastic effect of laser radiation and fast electrons was estimated by the percentage of the tumour growth inhibition and by the quantity of animals with the healed malignant neoplasms The data obtained were subjected to variation statistical treatment by the Student-Fisher method

laser radiation on these growths in series VII and VIII These malignant neoplasms of 9 to 12 mm diameter were exposed either to multiseance (as e g in series I—VI) irradiation by laser beams with an energy of 700 to 750 J, or to a single seance all surface action of 3 to 8 laser radiation pulses of the same energy with an interval of 5 to 10 min between each The most marked inhibition of the growth of the forestomach planocellular carcinoma and Harding Passy melanoma at a slightly smaller total dose of energy took place in the single seance all surface irradiation of tumours The difference in weight of malignant neoplasms in the groups of animals exposed to multi or single seance all surface irradiation was statistically significant ($p < 0.05$—0.02) (Table 2)

The test data obtained, as well as the above mentioned information in the literature, indicated the selective action of the laser beams on the pigmented tumours The influence of laser radiation in combination with fast electrons on the growth of malignant neoplasms was therefore investigated, the Harding Passy and B_{16} melanomas (series IX—XII) being employed The single seance all-surface irradiation of tumours was applied by 3 to 8 pulses of focussed laser beams with an energy of 700 to 750 J, depending on the size The results of these experiments summarized in Table 3 indicate that when the laser radiation is used in combination with fast electrons a more marked inhibition on growth is observed than when these effects are employed separately With the combined treatment of the tumours the number of mice with healing also increased (Fig 1) Thus, if the Harding Passy melanoma was exposed to the laser radiation only, the regression of the neoplasms never occurred, while when the laser beams and fast electrons were combined, the healing of the Harding-Passy melanoma took place in 2 out of 9 mice (series IX) and in 1 out of 9 mice (series X), the animals being observed during 3 to 5 months after irradiation. In the experiments with the B_{16} melanoma the influence of laser radiation produced the regression of the tumour only in 1 out of 9 animals of series XII At the same time, the combined irradiation of B_{16} melanoma by laser beams and fast electrons resulted in complete healing in 5 out of 9 mice of series XI and in 4 out of 9 animals of series XII

Discussion

The data presented in this report demonstrate that the antitumour effect of laser radiation depends on many factors of both biologic and physical natures The earlier investigations on the greater sensitivity of pigmented neoplasms to laser radiation (MINTON et coll 1965 a, GOLDMAN 1967 a, PIRUZYAN et coll 1967 a, KAVETSKY et coll 1969 b) have been confirmed This is associated with



Fig. 1. Harding-Passy melanoma. a) Before irradiation. b) Twenty four hours after irradiation by 5 pulses of 700 J neodymium laser (energy density 1 800 J/cm^2) and a single irradiation by high speed electrons in a dose of 1 000 rad (90 to 100 rad/min, 22 MeV). c) Five months after irradiation of tumour by laser beams and fast electrons in a cumulative dose of 6 000 rad. Tumour replaced by a smooth pigmented scar.

A more distinct antiblastic action of laser radiation was obtained while treating the forestomach planocellular carcinoma, which in contrast to the Ehrlich tumour has a 'softer' consistence, as well as the Harding-Passy melanoma. These malignant neoplasms were irradiated every other day by 1 to 2 pulses of laser beams, the energy of which was 700 to 750 J (Table 1).

Taking into account a relatively high sensitivity to the laser beams of the forestomach planocellular carcinoma and Harding-Passy melanoma, an attempt was made to reveal the influence of the irradiation rhythm on the antitumour effect of

Laser radiation with a modulated quality factor, when the pulse duration is measured in nanoseconds, exerts a greater destructive action upon the tumour than when it is in milliseconds or microseconds (GOLDMAN et coll 1964 b, MINTON & KETCHAM 1964, MINTON 1966 b)

The encouraging experimental results obtained induced a number of investigators to apply laser radiation in the treatment of human malignant tumours McGUFF et coll (1964, 1965, 1966 a) and McGUFF (1966 b, c) reported on the positive results of using ruby laser radiation in treating patients with malignant melanomas of different localizations and their subcutaneous and skin metastases as well as in recurrences that may appear after roentgen or surgical treatment The laser beams effect resulted in the diminution or even complete healing However, viable tumour cells in the zone of irradiation were often detected at histologic examination

Ruby or neodymium laser radiation was employed in the treatment of melanomas in man and their relapses and metastases in the skin as well as into subcutaneous cellular tissue (GOLDMAN et coll 1964 c, 1965, 1967, GOLDMAN 1966 a, c, 1967 a) These were essentially large tumours whose treatment by universally adopted methods would probably have proved unsuccessful, despite the satisfactory temporary result expressed in their complete disappearance in many cases, recurrence occurred after 6 to 8 months HELSPER et coll (1964) also observed healing of some of the subcutaneous nodes of the melanoma when they were exposed to radiation of ruby laser

Encouraging results of treating melanomas of essentially small size, their metastases and skin infiltration by neodymium laser radiation have been reported by KAVETSKY (1971), LAZAREV et coll (1971), LAGUNOVA et coll (1971) Favourable results were also obtained while irradiating the multiple pigmented and nonpigmented basal cell epitheliomas, malignant lymphomas, highly differentiad planocellular carcinomas of the orbit and of the maxillary sinus, the haemorrhagic Kaposi s sarcoma and mycosis fungoides (GOLDMAN et coll 1963 b, 1965, GOLDMAN & WILSON 1964, McGUFF 1966 b, c)

Laser beams were used with definite success in the treatment of planocellular carcinoma of the larynx, metastasis of a bronchial carcinoma into soft tissues, multiform glioblastoma, adenocarcinoma of the posterior wall of the rectum, epidermoid carcinoma of the glans penis (ROSOMOFF et coll 1965, McGUFF 1966 b, PARSONS et coll 1968) Along with the treatment of malignant neoplasms KAVETSKY (1971) and LAZAREV et coll (1971) are extensively applying the laser radiation to the therapy of such surface conditions of a benign and pretumour character as papillomas, nevi, angiomas, hemangiomas, fibromas, angiofibromas

the fact that the degree of laser beam absorption depends on the colouring of irradiated objects The pigmented cells of malignant and normal tissues absorb the laser radiation to the greatest degree (GOLDMAN et coll 1963 a, 1964 a, FINE et coll 1965, ROUNDS 1965, ROUNDS et coll 1965 a, KAVETSKY & GAMALEYA 1968). Apparently, the selectivity of the melanomas relative to laser radiation should be explained by the fact that for their destruction (all other things being equal) a much lesser dose of radiation is necessary than, e g for the non pigmented epithelium tumours and sarcomas (KETCHAM & MINTON 1965, KLEIN et coll 1965, GOLDMAN 1966 b)

Together with the contents of melanin, the microscopic structure and physical density of malignant neoplasms exert a considerable influence upon the degree with which the destructive action of the laser beam is manifested A substantial antitumour effect when acting on neoplasms of soft consistence is observed, this comprises a *small quantity of collagen intercellular substances, amply vascu* larized The malignant neoplasms of compact consistence with a large content of connective tissue elements of the struma are resistant to the action of laser beams Thus, the softer consistence of the planocellular carcinoma of the forestomach made it react more vigorously than the more compact Ehrlich tumour

Dependence exists between the *antitumour effect of the laser radiation* and the size of malignant neoplasms, i e their accessibility to the direct action of laser beams Small near-surface located tumours are more susceptible to laser therapy The regression of malignant neoplasms while being irradiated is limited by the zone of the direct action of laser radiation Parts of tumours which have not been irradiated possess the potency of progressive growth This suggests that the obligatory condition of success is the irradiation of the whole surface of malignant neoplasms, preferably at one session

Among the physical characteristics of laser radiation affecting the degree of tumour destruction, the value of pulse energy and (which is even more important) the energy density of the radiation, i e the value of energy flow per unit of irradiated surface, should be essentially noted A marked antiblastic effect takes place only with radiation of high energy laser, on the other hand the action of low energy laser beams (*especially on nonpigmented tumours*) usually fails to result in the destruction of malignant neoplasms (FINE et coll 1964 b, HELSPER et coll 1964, KLEIN et coll 1965, GOLDMAN 1965, 1966 b, KETCHAM & MINTON 1965, KOZLOV et coll 1971)

The wavelength of the laser radiation and the pulse duration also influence the antitumour *effect of laser beams The structural elements* of malignant and normal cells absorb more intensely the laser radiation of short wavelength, especially when it lies within the blue-green and ultra-violet regions of the spectrum (ROUNDS et coll 1965, HYME et coll 1966, MINTON 1966 a)



Fig 2 Harding Passy melanoma 1 hour after irradiation by one pulse of 700 j neodymium laser (energy density 18 000 j/cm²) Viable cells within necrotized tissue H & E x 120

cells of pigmented malignant neoplasms (LEONOV & SHKODYREV 1966, PIRUZYAN et coll 1971, KIRICHINSKY et coll 1971) When the malignant neoplasms are exposed to laser radiation of high peak energy (while the role of kinetic factor considerably increases), the propagation of tumour process may take place due to the introduction of the remaining viable blastoma cells into the lymph and blood vessels or into the normal tissues surrounding the tumour (GOLDMAN 1966 b, 1967 b, 1970, KETCHAM et coll 1967, 1970, MULLINS et coll 1967, HOYE et coll 1968)

The essential disadvantage of laser application is the difficulty of dosage, the energy varies considerably from pulse to pulse and this affects the results of the experiment

As the application of laser radiation experimentally as well as clinically did not always provide complete healing and as a certain time after irradiation recurrence was sometimes evident, attempts to increase the antiblastic action of laser beams were undertaken For this purpose dyes absorbing the energy of radiation in the red region of the spectrum such as, e g India ink, trypan blue, Evans blue, methylene blue, Janus green, introduced either parenterally or directly into the tumour node were used (ROSOMOFF & CARROLL 1966, LICHTENSTEIN 1968, KAVETSKY et coll 1970) The enhancement of the antitumour effect also took place when laser radiation was applied in combination with chemotherapy (MINTON et coll 1965 b, HOYE & WEISE 1966, HOYE et coll 1966, LICHTENSTEIN & BYALIK 1969, KAVETSKY et coll 1970) or such kinds of ionizing radia-

Clinical observations. The present authors in collaboration with I. M. HATCHATURYAN have treated 14 patients who had 85 different skin tumours. The source of the powerful laser radiation (Пульсар 1000 Pulsar-1000) was specially intended for oncologic purposes. The latter is a solid state free running pulsed glass neodymium laser supplied with an optical system for directing and focussing the beam onto the irradiated object.

The number of pulses incident upon the tumours ranged from 1 to 25 or more depending on their size. The pretumour conditions and benign neoplasms (papillomas, nevi, angiomas, angiofibromas, fibromas, keratomas) were exposed to laser radiation, the energy density of which varied from 200 to 450 j/cm. For the treatment of the skin the laser radiation of 400 to 500 j/cm energy density was used and, for the treatment of melanomas, a radiation with an energy radiation of 1 000 to 1 500 j/cm^2.

The effect of laser radiation produced complete healing of all tumours. The patients have been under observation for 1 to 6 months and no recurrences have been observed.

Results of clinical and experimental observations. The results of experimental research and clinical observations indicate laser therapy as a new method of tumour treatment. Its distinctive feature is its capability of causing local necrosis of malignant neoplasms without injuring the surrounding normal tissues. Laser beams practically do not exert any unfavourable influence on the general condition of the organism, which makes them advantageously different from chemotherapeutic preparations and ionizing radiations (FINE et coll. 1964 b, PEREs et coll. 1964, KETCHAM & MINTON 1965, KAVETSKY et coll. 1969 a). Among the valuable features of laser therapy are also the painlessness, the absence of any side reactions, the favourable course of the irradiated focus healing usually unaccompanied by infection (GOLDMAN et coll. 1964 c, McGUFF 1966 b, KAVETSKY 1971).

It should, however, be borne in mind that the antiblastic effect may be connected with some negative factors in the laser radiation influence. Thus the histologic test of malignant neoplasms that had been exposed to laser radiation not seldom detects viable tumour cells in the necrotized malignant tissue (PIRUZYAN et coll. 1967 b, HOYE et coll. 1967 a). A microscopic analysis of Harding Passy melanoma one hour after its exposure to a pulse of focussed laser radiation with an energy of 750 j revealed viable tumour cells, both in singular and rosette type complexes, within the affected region, which was represented by a nonstructural mass of coagulated cells with numerous slits and lacunae of various sizes (Fig. 2.). The presence of viable tumour cells within the necrotic masses is apparently connected both with the nonuniform distribution of energy density along the cross section of the laser beam and with the irregular contents of melanin in the

ment of tumours in combination with chemotherapy or ionizing radiations seems to be promising

SUMMARY

It has been demonstrated in white nonbred mice as well as in mice of the line CC B_r and C $_7B_1$ that irradiation by neodymium laser caused destruction of Ehrlich tumours, fore stomach planocellular carcinomas Harding Passy and B_{16} melanomas The most sensitive to the laser beams were tumours with melanin contents and nonpigmented malignant neoplasms of 'softer' consistence than the Ehrlich tumour A considerable antitumour effect was observed only in the high energy laser irradiation of its whole surface preferably during a single session and was enhanced by fast electron irradiation Complete healing occurred of 85 benign and malignant neoplasms in 44 patients The results have remained most satisfactory during 6 months of observation

ZUSAMMENFASSUNG

Es ist bei weissen nicht inzucht Mausen sowie bei Mausen der Stamme CC_3 B_r und C_3 B_1 gezeigt worden dass Bestrahlung mit einem Neodymium Laser zum Untergang von Ehrl ch Tumoren planozellularen Karzinomen des Vormagens (SPC) Harding Passy und B_{16} Melanomen fuhrt Am empfindlichsten gegenuber Laser waren Tumoren mit einem Melanininhalt und nicht pigmentierte Neoplasmen weicherer Konsistenz als Ehrlich Tumoren Ein ausgesprochener Antitumoreffekt war nur bei hochenergetischer Laser Bestrahlung der gesamten Oberflache vorwiegend in einer Sitzung zu beobachten, und war durch Bestrahlung mit schnellen Elektronen erhoht Eine vollstandige Heilung trat bei 85 gutart gen und bosartigen Neoplasien bei 44 Patienten auf Diese Ergebnisse waren voll zufriedenstellend wahrend einer 6 monatigen Beobachtungszeit

RÉSUMÉ

Les auteurs ont montre sur des souris blanches de lignee impure ainsi que sur des souris de la lignee $CC_{37}B_r$ et $C_{37}B_1$ que l irradiation par le laser au neodyme detruit les tumeurs d Ehrlich le cancer planocellulaire de l estomac anterieur et les melanomes de Harding Passy et B_{16} Les tumeurs les plus sens bles aux rayons du laser sont les tumeurs qui contiennent de la melanine et les néoplasies malignes non pigmentees de consistance « plus molle » que la tumeur d Ehrlich Ils n ont observé un effet anti tumoral important qu avec l irradiation par un laser de haute énergie de toute la surface tumorale de preference en une seule seance son effet est augmente par l irradiation avec des electrons rapides La guerison complete de 85 neoplasies benignes et malignes a ete obtenue sur 44 sujets Les resultats sont restes tres satisfaisants au cours d une observation de 6 mois

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tions as roentgen and gamma radiation (McGuff et coll 1966 b, Helsper et coll 1966, 1967, Hoye et coll 1967, Kozlov et coll 1971) The present investigations into the treatment of malignant melanomas by laser radiation combined with fast electrons disclosed a more distinct antiblastic effect than that obtained when the tumours were irradiated either by laser beams or by electrons separately.

The fast electrons were chosen from ionizing radiations because, owing to their physical properties, they have a number of advantages over other kinds of ionizing radiations in the treatment of surface neoplasms (Tubiana et coll 1963), i e for those localizations where at present the use of laser is possible The antiblastic effect observed while exposing the melanomas to laser radiation and to fast electrons, possibly depends, as in laser beam combinations with roentgen radiation or gamma rays (^{60}Co) on the phenomenon of synergism in the action of each of the agents used

Conclusion

The experimental data presented in this report convincingly demonstrate the capability of laser radiation of destroying malignant neoplasms The most sensitive to the laser beams are those with melanin contents as well as the non-pigmented malignant neoplasms of 'soft' consistence An obligatory condition of successful laser therapy should be the exposure of the whole surface of the growth to the high-energy radiation, preferably during a single seance The antitumour effect of laser radiation may be enhanched by means of ionizing radiation, such as fast electrons

As regards the application of laser radiation in the oncologic clinic, it is now obvious that it cannot take the place of (as previously suggested by some investigators) the usual methods of the treatment of malignancy while its employment is limited solely to surface neoplasms It is most expedient to use the laser beams for the treatment of localized forms of human tumours of a precancerous and benign character The data which are to be found in the literature indicate the possibility of employing this radiation in the therapy of malignant neoplasms of human skin It is essential to conduct further research in a clinical direction and to elaborate the indications for laser therapy in surface (skin deep) tumours The widening of its application in oncology may be obtained by powerful clinical laser installations as well as lasers with a beam wavelength that can be smoothly readjusted from the ultraviolet to infrared regions of the spectrum The building up of fibre optics that could withstand the high energy laser radiation would also enable the potentialities of applying the laser in oncology to be increased The application of the radiation in the treat-

ment of tumours in combination with chemotherapy or ionizing radiations seems to be promising

SUMMARY

It has been demonstrated in white nonbred mice as well as in mice of the line CC$_{57}$Br and C$_{57}$Bl that irradiation by neodymium laser caused destruction of Ehrlich tumours fore stomach planocellular carcinomas, Harding Passy and B$_{16}$ melanomas The most sensitive to the laser beams were tumours with melanin contents and nonpigmented malignant neoplasms of 'softer' consistence than the Ehrlich tumour A considerable antitumour effect was observed only in the high energy laser irradiation of its whole surface, preferably during a single session and was enhanced by fast electron irradiation Complete healing occurred of 85 benign and malignant neoplasms in 44 patients The results have remained most satisfactory during 6 months of observation

ZUSAMMENFASSUNG

Es ist bei wei [illegible] und C$_{57}$B$_1$ gezeigt w [illegible] von Ehrlich Tumore [illegible] und B$_{16}$ Melanomen zu it Am empfindlichsten gegenuber Laser waren Tumoren mit einem Melanininhalt und nicht pigmentierte Neoplasmen ‚weicherer' Konsistenz als Ehrlich Tumoren Ein ausgesprochener Antitumoreffekt war nur bei hochenergetischer Laser Bestrahlung der gesamten Oberflache vorwiegend in einer Sitzung, zu beobachten, und war durch Bestrahlung mit schnellen Elektronen erhoht Eine vollstandige Heilung trat bei 85 gutartigen und bosartigen Neoplasien bei 44 Patienten auf Diese Ergebnisse waren voll zufriedenstellend wahrend einer 6 monatigen Beobachtungszeit

RÉSUMÉ

Les auteurs ont montre sur des souris blanches de lignee impure ainsi que sur des souris de la lignee CC$_{57}$Br et C$_{57}$Bl que l irradiation par le laser au neodyme detruit les tumeurs d Ehrlich le cancer planocellulaire de l estomac anterieur et les melanomes de Harding Passy et B$_{16}$ Les tumeurs les plus sensibles aux rayons du laser sont les tumeurs qui contiennent de la melanine et les neoplasies malignes non pigmentees de consistance « plus molle » que la tumeur d Ehrlich Ils n ont observe un effet anti tumoral important qu avec l irradiation par un laser de haute energie de toute la surface tumorale, de preference en une seule seance, son effet est augmenté par l irradiation avec des electrons rapides La guerison complete de 85 neoplasies benignes et malignes a ete obtenue sur 44 sujets Les resultats sont restes tres satisfaisants au cours d une observation de 6 mois

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DOSIMETRIC PROBLEMS WITH RADIUM IN THE INTRACAVITARY TREATMENT OF CARCINOMA OF THE UTERINE CERVIX

J E JOHNSSON and ULLA BRITA NORDBERG

Intracavitary irradiation of the primary tumour is generally included in the radiation therapy of carcinoma of the uterine cervix An intrauterine applicator in the form of a rod and one or several vaginal applicators are loaded with radioactive isotopes The form of the rod is about the same regardless of the technique employed but the vaginal applicators vary The Stockholm technique employs at flat or slightly curved applicator (KOTTMEIER 1964), the Paris and Manchester methods utilize ellipsoids or cylinders placed in the vaginal vaults and sometimes against the portio (TODD & MEREDITH 1953, FLETCHER 1966)

A number of means have been suggested for overcoming the difficulties inherent in ensuring that the applicators remain in position and in correct interrelationship, these include locking them together or fixing them so that only unimportant variations of positions are possible (NOLAN 1962, HENSCHKE & HILARIS 1965, SCOTT 1966, SCOTT & BETSCH 1966, CAMPBELL & DOUGLAS

Submitted for publication 28 December 1972

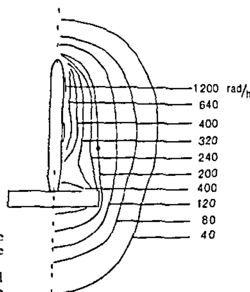


Fig 1 Isodose distribution around the intrauterine and vaginal applicators in ideal position The numbers represent the absorbed dose in rad/h Point A, marked by a dot, lies 2 cm laterally and 2 cm caudally to the point of contact between the intrauterine and vaginal applicators

1966, LOISSEAU 1967). The purpose of the present investigation has been to demonstrate the importance of a well-defined fixed geometry for the intrauterine and vaginal applicators, and to present a method for its achievement

Material and Methods The intracavitary Stockholm method was employed A rod-shaped applicator is placed in the uterus and a flat applicator fixed against the portio with gauze packing The most commonly used applicators were examined *Intrauterine applicator* ∅ *7 mm × 68 mm,* 90 mCi ²²⁶Ra Vaginal applicator 5 mm × 44 mm × 44 mm, 110 mCi ²²⁶Ra

The resultant isodose distribution in water around these applicators, combined in four different geometries, was calculated (1) 'Ideal position', that is the intrauterine applicator is placed orthogonally in direct contact with, and in the centre of the principal plane of the vaginal applicator (Fig 1) (2) 10° and (3) 20° tilt from normal, parallel to one side of the intrauterine applicator (4) The intrauterine applicator in an orthogonal position but at 1 cm distance from the vaginal applicator

A point (A, Fig 1) was chosen as the point of reference for indicating the absorbed dose, this lies 2 cm cranially and 2 cm laterally from the point of contact of the intrauterine and vaginal applicators (approximately at the external os of the cervix)

The dose at point A was calculated from films obtained during the actual treatment in 7 consecutive cases without any interfixation of the applicators

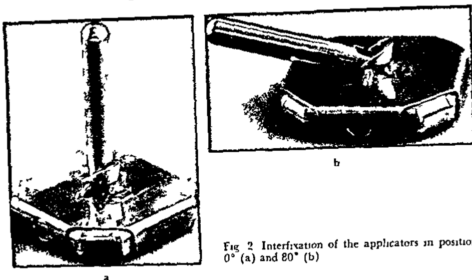


Fig 2 Interfixation of the applicators in position 0° (a) and 80° (b)

An arrangement for this purpose was later constructed (Fig 2) A thin metal frame was drawn over the vaginal applicator and the intrauterine applicator, mounted with a cross-piece, fitted into a notch in the frame so that it could be moved through an angle of incidence only of 0° to 80° in a direction parallel to the side of the vaginal applicator A catch prevented movements beyond the normal position (0°) in the opposite direction (Fig 2) Frontal and lateral films were obtained in 47 patients during actual treatment in which the locking arrangement was employed

Results

The isodose distribution around the combination of applicators in the ideal position (Fig 1) indicates a steep dose gradient An absorbed dose which at point A was 6 500 rad had at only 5 mm lateral to the point sunk to 5 000 rad

Inclination of the intrauterine applicator 10° from normal deforms the isodose pattern so that the absorbed dose at the two points A given above for the ideal position is increased to 8 000 and decreased to 5 000 rad, respectively, for the same treatment time An inclination of 20° from normal gives corresponding absorbed doses of 12 000 and 3 500 rad, respectively If the lower end of the intrauterine applicator is withdrawn 1 cm from the vaginal applicator, a waist arises in the isodose pattern, and the dose at point A falls to 3 500 rad

Calculations of the absorbed dose in 7 patients in whom the applicators were not interlocked disclosed 3 600 to 6 800 rad, mean value 4 900 rad, at point A

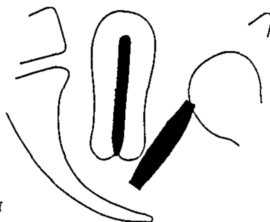


Fig. 3. Applicators inserted without interfixation. The vaginal applicator has almost slid off the portio.

on the side on which the dose was lowest, and 6 200 to 14 600 rad, mean value 8 500 rad, on the side where the dose was highest. The applicators failed to be in the ideal position even if the deviations were not so great as depicted in Fig. 3, a calculation of the dose had not been performed in this instance. The treatment period for these 7 patients gave about 6 500 rad at point A with ideal positions of the applicators.

The applicators with the locking arrangement described are inserted at 80° and the bar is turned ventrally. When the vaginal applicator is lightly pressed against the portio above, the uterus usually becomes slightly anteflexed and the intrauterine applicator is locked in position at 0°. Where the portio is not prominent and the uterus lies retroflexed, it is sometimes necessary to rotate the combination half a turn after insertion so that the bar is turned dorsally. The uterus may then be straightened into middle position to light anteflexion. If the intrauterine and vaginal applicators can be inserted individually, that is when the space in the vagina is sufficient, the applicators may also be interlocked in the manner described.

Roentgenograms obtained at actual treatments indicate that the angle between the intrauterine and vaginal applicators always deviates under 10° from the ideal position.

Discussion and Conclusion

The distribution of the absorbed dose around the combination of applicators has an extremely steep gradient. Only primary tumours lying schematically within a hemispheric volume with a radius of 2 cm around the external o of the cervix can be expected to be eliminated by the doses indicated (6 500 rad at point A). Calculation of the dose distribution around the applicators in

different positions in relation to one another revealed that even quite small variations of these positions cause marked changes in the distribution of the dose When the applicators were individually placed in the uterus and the vagina, their interrelation positions varied to a high degree (UNNERUS et coll 1964, SCOTT & BETSCH 1966, LOISSEAU 1967) Such alterations might lead to parts of the tumour receiving too small a dose, and the bladder or intestines given excessive irradiation

The intrauterine and vaginal applicators must be interfixed in a predetermined position in order to guarantee an adequate distribution of the dose around the portio—cervix (Fig 1)

The coupling arrangement described is uncomplicated and allows the use of applicators already available, so that it is possible to work with the distribution of dose (theoretically) and the dose levels already in use

The investigation indicates that the relative positions of the intrauterine and vaginal applicators with the coupling used are almost ideal The arrangement has been in routine use in our clinic for four years

SUMMARY

The distribution of dose around intracavitary applicators (Stockholm technique) has been investigated Both applicators must be fixed in predetermined positions in order partly to guarantee a satisfactory distribution of dosage in the area of the tumour, and partly to protect the bladder and intestines An uncomplicated but effective coupling arrangement for such fixation is presented

ZUSAMMENFASSUNG

Die Dosisverteilung um intrakavitare Applikatoren (Stockholm Techn [illegible]
sucht Beide Appl [illegible]
eine zufr [illegible]
und Darr [illegible] e, doch effektive Kupplungsanordnung fur diese
Fixation wird angegeben

RÉSUMÉ

Les auteurs ont etudie la distribution de dose autour d applicateurs intracavitaires (technique de Stockholm) Les deux applicateurs doivent etre fixes dans des positions predeterminees de façon a garantir une distribution satisfaisante de la dose dans le volume tumoral d une part et de façon à protéger la vessie et l intestin d autre part Les auteurs presentent un dispositif de couplage simple mais efficace pour cette fixation

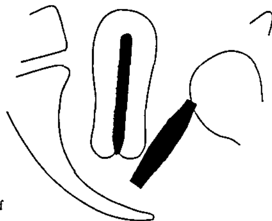


Fig 3 Applicators inserted without interfixation The vaginal applicator has almost slid off the portio

on the side on which the dose was lowest, and 6 200 to 14 600 rad, mean value 8 500 rad, on the side where the dose was highest The applicators failed to lie in the ideal position even if the deviations were not so great as depicted in Fig 3, a calculation of the dose had not been performed in this instance The treatment period for these 7 patients gave about 6 500 rad at point A with ideal positions of the applicators

The applicators with the locking arrangement described are inserted at 80° and the bar is turned ventrally When the vaginal applicator is lightly pressed against the portio above, the uterus usually becomes slightly anteflexed and the intrauterine applicator is locked in position at 0° Where the portio is not prominent and the uterus lies retroflexed, it is sometimes necessary to rotate the combination half a turn after insertion so that the bar is turned dorsally The uterus may then be straightened into middle position to light anteflexion If the intrauterine and vaginal applicators can be inserted individually, that is when the space in the vagina is sufficient, the applicators may also be interlocked in the manner described

Roentgenograms obtained at actual treatments indicate that the angle between the intrauterine and vaginal applicators always deviates under 10° from the ideal position

Discussion and Conclusion

The distribution of the absorbed dose around the combination of applicators has an extremely steep gradient Only primary tumours lying schematically with a hemispheric volume with a radius of 2 cm around the external os of the cervix can be expected to be eliminated by the doses indicated (6 500 rad at point A) Calculation of the dose distribution around the applicators in

FROM THE DEPARTMENT OF DIAGNOSTIC RADIOLOGY (DIRECTOR PROF M BUSCH), MEDIZINISCHE POLIKLINIK, AND KLINIK FUR STRAHLENTHERAPIE UND RONTGEN-DIAGNOSTIK (DIRECTOR PROF F HESS), UNIVERSITY OF MARBURG, 355 MARBURG AN DER LAHN, WEST GERMANY

OPTIMIZATION OF RADIUM SOURCES FOR VAGINAL TREATMENT

M BUSCH and L BUCHELT

A computer examination of dosimetry and dosage in interstitial and intracavitary therapy was commenced in 1962 Computer-calculated absorbed doses and absorbed dose rates of gamma rays around radium (LAUGHLIN et coll 1963, ADAMS & MEURK 1964, HOPE et coll 1964, BUSCH 1964) possess a high clinical degree of reliability and accuracy Our latest efforts constitute an attempt to improve the work of optimization of radium therapy initiated by PATERSON & PARKER (1934)

Local irradiation of the vagina usually follows total hysterectomy in carcinoma of the uterus Calculations of commercial radium preparations for vaginal treatment appear to present irregularities in the dose rate at a 5 mm depth of tissue of 40 to 50 per cent It is probable that vaginal strictures and a spread of the malignancy behind these may be caused by such irregularity in dosage (FLETCHER & STOVALL 1962)

Many dose distributions of radium for vaginal treatment have recently been calculated with a numerical procedure termed an 'envelope integral' (BUSCH

Submitted for publication 26 June 1972

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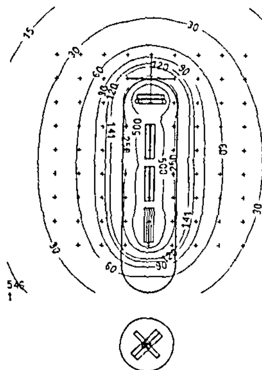


Fig 1

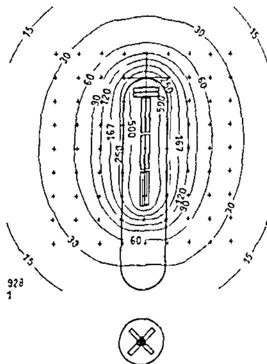


Fig 2

Fig 1 Radium cylinder for vaginal application optimized by a full automated procedure in the computer programme Isodose curves, cylinder and radium tubes plotted to normal scale with matrix points at 1 cm distances Isodoses in rad per hour Charge $3\times10+2\times10+2\times10+3\times10$ mg radium, an impractable arrangement

Fig 2 Radium cylinder 20 mm in diameter for practical vaginal application Charge $2\times10+2\times10+2\times10+3\times10$ mg radium

1967). Several of these radium arrangements produced good uniformity of dose rate at 5 mm tissue depth, i e 5 mm distance from the surface of the cylinder containing the radium tubes A fully automated optimization procedure in the computer programme was then developed, this calculated optimal distances between the single radium groups to produce a dose rate most uniform at 5 mm tissue depth This, however, required the construction of special cylindric forms for many optimized radium arrangements or an impractical charging technique

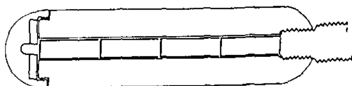


Fig 3 Plastic cylinder for about 20 different optimized radium arrangements

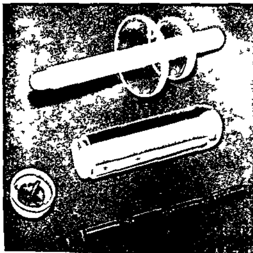


Fig 4 Plexiglas cylinder 20 mm in diameter with a spherical cap for two cross radium tubes with four nickel cartridges and a plexiglas handle and two variable disks

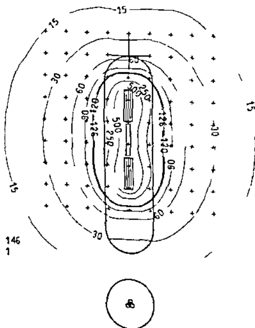


Fig 5 Radium arrangement for the treatment of vaginal tumours or metastases Cylinder 23 mm in diameter Charge $3 \times 10 + 1 \times 10 + 3 \times 10$ mg radium

(Fig 1), only those methods are suitable for clinical use that allow a simple technique as well as a sufficient uniformity of dose rate The adequacy of uniformity was the deviation of the relevant isodose curve from the 5 mm tissue depth line, this deviation proved to be less than 0 5 mm (Fig 2)

A plexiglas cylinder with a central bore and suitable spherical caps appears in Fig 3 The cap may be charged with two, three or four radium tubes, arranged criss-cross, 15 mm in length and 2 mm in diameter, 0 3 mm Pt-filtration and 10 mg radium The bore of the cylinder may be filled with four or less different groups of the same radium tubes, each group in a thin nickel cartridge, these touch and only in a few special instances are interdistances of 5 or 10 mm allowed The bore of the cylinder is occluded by a plexiglas handle (Fig 4)

The 'Marburger Radium Applikatoren Set' will consist of three cylinders of 20, 23, and 26 mm diameter, each with two different caps, the smallest cylinder is already in clinical use Each cylinder is sufficient for about 20 different optimized radium assemblies with dose rates from 140 to 200 rad per hour at 5 mm tissue depth The arrangement of the radium within the bore of the cylinder also enables the radiation treatment of localized metastases in the vagina (Fig 5) The technical realization of optimized eccentric radium arrangements thus appears to have been reached

SUMMARY

A means of vaginal radium treatment was developed that enabled several optimized radium arrangements for a uniform dose distribution at 5 mm tissue depth The charging technique and clinical application have proved to be simple

ZUSAMMENFASSUNG

Ein Verfahren zur vaginalen Radiumbehandlung, die verschiedene optimal angepasste Radiumanordnungen fur eine einheitliche Dosisverteilung in einer *Gewebstiefe von 5 mm* ermoglicht, wurde entwickelt Die Ladungstechnik und die klinische Anwendung erwiesen sich als einfach

RÉSUMÉ

Les auteurs ont mis au point une technique de traitement intravaginal par le radium qui permet diverses dispositions optimales du radium pour une distribution de dose *uniforme* à une profondeur de 5 mm dans les tissus La technique de chargement et l'application clinique se sont revelees simples

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APPLICAZIONI RICERCHE SCIENTIFICHE, ISTITUTO DI SCIENZE RADIOLOGICHE (PROF A PERUSSIA), UNIVERSITÀ DEGLI STUDI, ET ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (PROF A BANFI), MILAN, ITALIE

DOSIMÉTRIE PAR ORDINATEUR EN CURIETHÉRAPIE

G DONATI et F. VOLTERRANI

La connaissance de la répartition dans l'espace de la dose absorbée, jointe à une évaluation critique de l'extension clinique des lésions constitue la condition essentielle pour une bonne réussite de tout traitement par les radiations et représente l'étape indispensable pour aboutir à la réalisation d'un plan de traitement correct

Dans la curiethérapie, la distribution de la dose est inévitablement assez complexe et différente suivant les cas, même à égalité de modalités techniques, d'où la difficulté de l'étude dosimétrique

On a proposé récemment de nouvelles méthodes de traitement à préparation non radioactive permettant d'étendre utilement les indications de la curiethérapie et d'améliorer sensiblement les conditions de protection du personnel (CHASSAGNE et coll 1966, 1969, DELOUCHE et coll 1968, HENSCHKE et coll 1963, PIERQUIN 1966) A cause de l'originalité de leur technique, et des différentes substances radioactives employées, notamment ^{192}Ir et ^{137}Cs, elles s'écartent sensiblement des techniques habituelles au radium

Il est naturel, par conséquent, de considérer comme plus juste de n'en affronter l'étude et l'application, à défaut de l'expérience découlant de la pratique, que si l'on est en mesure de vérifier d'une façon adéquate les conditions d'irradiation réalisées avec ces méthodes à travers une évaluation dosimétrique raffinée Ceci impose d'ailleurs une vérification de la dosimétrie dans la radiumthérapie, qui est loin d'être résolue d'une manière adéquate par les méthodes

Soumis à la Rédaction le 14 mars 1972

habituelles, convaincus que nous sommes de l'utilité de conserver comme paramètre guide, en affrontant de nouvelles méthodes, la confrontation avec des techniques dont les résultats positifs obtenus ont désormais largement fait leurs preuves, depuis près d'un demi siècle, sur le plan clinique.

La mesure directe de la dose pourrait, par elle-même, représenter la solution la plus satisfaisante. Cependant, malgré les gros progrès réalisés dans ce domaine, les difficultés techniques que l'on rencontre, liées au volume et à la sensibilité des appareils de mesure, n'en rendent possible et utile l'application à la pratique clinique que dans certaines circonstances seulement.

La méthode du calcul est, en définitive, la plus appropriée en curiethérapie pour atteindre les buts fixées.

Toutes les méthodes de calcul partant de reconstructions, aussi bien graphiques que matérielles des implantations, sont longues et délicates, malgré la simplicité apparente des opérations élémentaires, et présentent bien des possibilités d'erreur. Seul le calcul direct à partir des conditions d'irradiation que l'on réalise pour chaque cas en particulier, peut fournir les informations indispensables et utiles.

Bon nombre d'auteurs ont démontré l'utilité de l'emploi des ordinateurs pour résoudre les problèmes dosimétriques posés par la radiothérapie et, en particulier, par la curiethérapie et de nombreux programmes de calcul ont été publiés (ADAMS et coll. 1965, BALTER 1969, JAMESON et coll. 1968, LAUGHLIN et coll. 1968, POWERS et coll. 1965, ROSE et coll. 1966, ROSENWALD et coll. 1970, SHALEK et coll. 1968, TSIEN 1955). Ils ont permis d'effectuer une étude dosimétrique toujours plus approfondie des différentes techniques curiethérapeutiques connues, une évaluation des nouvelles méthodes sur des bases plus rationelles, ainsi que le développement de nouveaux schémas théoriques de traitement (DURRANCE et coll. 1968, DUTREIX et coll. 1968, PAINE et coll. 1969, PIERQUIN et coll. 1968, 1969, SCHLIENGER et coll. 1970). Les informations recueillies, en outre, se sont avérées susceptibles d'influencer positivement les décisions cliniques quant à la distribution des sources et à la durée du traitement (CASTRO et coll. 1969, CHASSAGNE et coll. 1970, FLETCHER et coll. 1962).

Nous avons donc jugé opportun de nous consacrer à l'étude d'un programme pour le calcul par ordinateur de la distribution dans l'espace de la dose en curiethérapie, et, n'estimant pas tout à fait adéquates les solutions déjà proposées, nous nous sommes efforcés d'obtenir de meilleurs résultats.

Dans notre travail, nous nous sommes proposé de *réaliser un instrument* valable, d'un emploi simple et le plus utile possible. Tout d'abord, nous nous sommes préoccupés de satisfaire deux exigences fondamentales, à savoir : l'étude et le projet des dispositions géométriques des sources [illegible]

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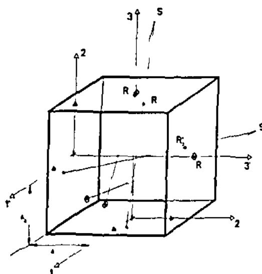


Fig 1 Schéma du dispositif porte cassettes

Dans l'organisation des données de « entrée » et de « sortie » nous nous sommes efforcés de laisser la plus grande liberté possible à l'utilisateur, compte tenu des exigences cliniques

Dans la version plus complète, la séquence des opérations gérées par le programme comprend (1) l'identification de la géometrie du traitement et des plans de calcul, (2) l'organisation du calcul en vue d'obtenir une représentation des résultats sous forme de diagramme d'isodoses, (3) le calcul de la dose

Dans ce qui suit, on décrira, de façon détaillée, les opérations effectuées et la méthode de calcul utilisée et l'on presentera, en outre, certains exemples de calculs

Identification de la géométrie du traitement et des plans de calcul

Repérage des sources radioactives

Dans cette étape, nous avons repris et développé le principe de repérage des foyers radioactifs en curiethérapie au moyen de radiographies orthogonales (HIDALGO et coll 1967, ROSENWALD et coll 1970) en apportant les modifications qui nous ont semble opportunes, en vue d'éliminer les sources d erreur liées à une technique radiographique imparfaite

La région anatomique, siege du dispositif d irradiation, est radiographiée, dans les deux projections orthogonales, en utilisant un porte-cassettes de type special, en plexiglas (Figs 1, 2) Sur les côtes du porte-cassettes, en contact avec les cli-

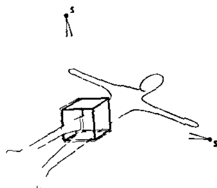


Fig 2 Schema des conditions de reperage des sources

chés sont visualisés en plomb deux systèmes de coordonées qui, radiographiés sur les films, faciliteront l'identification des points nécessaires pour le calcul Sur les côtes opposés aux cliches il existe une paire de marques plombées fixes, principale et secondaire, (R_1—R_2, R_1'—R_2') La marque principale (R_1) est alignée avec l origine du systeme de coordonnées reproduit sur le côté du porte-cassettes en contact avec le film, sur une même droite perpendiculaire à ce dernier

La disposition des marques principales et secondaires étant connue, et le porte-cassettes assurant aux deux films une position rigoureusement orthogonale, il est possible de calculer la position des sources radiographiques, ainsi que la disposition reelle dans l'espace des foyers radioactifs et des reperes de référence choisis (anatomiques ou introduits artificiellement dans le malade)

De cette façon la validité des résultats est indépendante de la position du malade par rapport au systeme de repérage et n'est pas influencée par la position ou par l inclinaison des faisceaux radiographiques

Calcul de la position reelle dans l'espace des sources radiographiques S et S' Pour chacune des sources radiographiques, il est possible d'ecrire six equations reliant entre elles les coordonnées des marques fixes, les coordonnées des sources, ainsi que les coordonnées des images des deux marques, relevées sur chacun des deux films

$$\frac{XR_{i,1} - YA_{i,1}}{XS_1 - YA_{i,1}} = \frac{XR_{i,2} - YA_{i,2}}{XS_2 - YA_{i,2}} = \frac{XR_{i,2}}{XS_2} \quad i = 1,2 \qquad (1)$$

avec

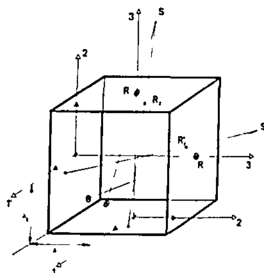


Fig. 1. Schéma du dispositif porte-cassettes.

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La région anatomique, siège du dispositif d'irradiation, est radiographiée, dans les deux projections orthogonales, en utilisant un porte-cassettes de type spécial, en plexiglas (Figs. 1, 2). Sur les côtes du porte-cassettes, en contact avec les ch-

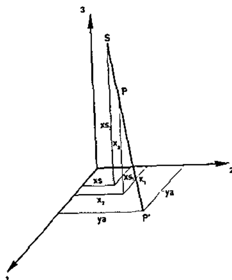


Fig 3 Image P donnee d un point P par la source rad ographique S sur le plan du film

C est ainsi que les calculs sont etablis dans un systeme de reference tridimen sionnel d axes cartesiens qui a pour origine un repere, anatomique ou artificiel, choisi pour chaque cas et independant des conditions de centrage ou bein un repere fixe et valable pour tous les malades et pour un meme type de traitement

La position de ce repere est calculee à partir des coordonnees de ses images, lues sur les deux radiographies orthogonales De façon analogue on identifie la position des reperes de reference accessoires, des sources radioactives ponctuelles et des extremites des sources lineaires

Semblablement a ce qui a ete effectue pour la determination de la position des sources radiographiques, il est possible d ecrire six equations reliant entre elles les coordonnées des sources radiographiques dans le systeme des deux cliches (XS_i et XS_i'), connues grâce au calcul precedent, les coordonnees des projections sur les deux films (YA_i et YA_i') du point dont on veut determiner la position dans l espace ainsi que les coordonnes inconnues de ce point (XS_i) (Fig 3)

$$
\begin{aligned}
f_1 &= (X_1 - YA_1)(XS_2 - YA_2) - (X_2 - YA_2)(YS_1 - YA_1) = 0 \\
f_2 &= (X_1 - YA'_1)(XS'_2 - YA'_2) - (X_2 - \delta_2 - YA'_2)(XS'_1 - YA'_1) = 0 \\
f_3 &= X_3 (XS_1 - YA_1) - XS_3 (X_1 - YA_1) = 0 \\
f_4 &= (X_2 + \delta_1)(XS'_1 - YA'_1) - XS'_3 (X_1 - YA'_1) = 0 \\
f_5 &= X_3 (XS_2 - YA_2) - XS_3 (X_2 - YA_2) = 0 \\
f_6 &= (X_3 + \delta_1)(XS'_2 - YA'_2) - XS'_3 (X_2 - \delta_2 - YA'_2) = 0
\end{aligned}
\qquad (5)
$$

$XR_{i,j}$ = coordonnée j de la marque i
XS_j = coordonnée j inconnue de la source
$YA_{i,j}$ = coordonnée j de l'image de la marque i sur le cliché

Même en tenant compte que les six équations ne sont pas toutes indépendantes entre elles, *le nombre des équations indépendantes dépasse* le nombre des inconnues. Pour la solution on applique donc la méthode des moindres carrés en écrivant (1) sous la forme :

$$f_i = (XR_{i,1} - YA_{i,1})(XS_2 - YA_{i,2}) - (XR_{i,2} - YA_{i,2})(XS_1 - YA_{i,1}) = 0 \ (i=1,2)$$
$$f_{i+2} = XR_{i,3}(XS_1 - YA_{i,1}) - XS_3(XR_{i,1} - YA_{i,1}) = 0 \ (i=1,2) \qquad (2)$$
$$f_{i+4} = XR_{i,3}(XS_2 - YA_{i,2}) - XS_3(XR_{i,2} - YA_{i,2}) = 0 \ (i=1,2)$$

La solution du système d'équations (2) correspond au minimum de la fonction

$$F = \sum_{1i}^{6} f_i^2 \qquad (3)$$

En posant la condition de minimum

$$\frac{\partial F}{\partial XS_j} = 0 \ (j=1,3) \qquad (4)$$

on obtient un système de trois équations linéaires par rapport aux trois inconnues XS_j.

Pour résoudre le système, on emploie la méthode de Gauss-Jordan qui s'avère stable du point de vue numérique, requiert un nombre peu élevé de calculs, et ne se ressent pas autant que d'autres méthodes des erreurs d'arrondi dans le cas où le déterminant de la matrice est petit.

Calcul des positions du repère de référence et des sources radioactives. Après avoir identifié la position dans l'espace des sources radiographiques on définit en premier un *repère fondamental, qui sert de référence pour la mise en place* des calculs. C'est par rapport à lui que sont définies les positions dans l'espace des *sources radioactives et de repères accessoires* (radio-opaques, anatomiques, osseux, ou introduits artificiellement dans le malade) servant à individualiser les *rapports du dispositif d'irradiation avec son anatomie.*

La définition du repère susdit est conforme au principe de ne pas être lié à des *choix préétablis*, mais plutôt à réserver la possibilité de mettre en relation, dans tous les cas, le calcul avec l'anatomie individuelle du sujet soumis au traitement.

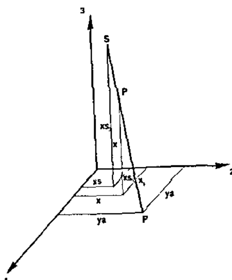


Fig 3 Image P donnee d un point P par la source radiographique S sur le plan du film.

C est ainsi que les calculs sont etablis dans un systeme de reference tridimensionnel d axes cartesiens qui a pour origine un repere anatomique ou artificiel, choisi pour chaque cas et independant des conditions de centrage ou bien un repere fixe et valable pour tous les malades et pour un meme type de traitement

La position de ce repere est calculee a partir des coordonnees de ses images, lues sur les deux radiographies orthogonales De façon analogue on identifie la position des reperes de reference accessoires des sources radioactives ponctuelles et des extremites des sources lineaires

Semblablement a ce qui a ete effectue pour la determination de la position des sources radiographiques il est possible d ecrire six equations reliant entre elles les coordonnees des sources radiographiques dans le systeme des deux cliches (XS et XS_i) connues grace au calcul precedent les coordonnees des projections sur les deux films (XA et YA_i) du point dont on veut determiner la position dans l espace ainsi que les coordonnes inconnues de ce point (XS) (Fig 3)

$$
\begin{aligned}
f_1 &= (X_1 - XA_1)(XS_2 - XA_2) - (Y_2 - YA_2)(YS_1 - YA_1) = 0 \\
f_2 &= (X_1 - XA_1)(XS'_2 - XA_2) - (Y_2 - \delta_2 - YA_2)(XS_1 - YA_1) = 0 \\
f_3 &= X_2(XS_1 - XA_1) - XS_2(Y_1 - YA_1) = 0 \\
f_4 &= (X_2 + \delta_1)(XS'_1 - XA_1) - XS_2(Y_1 - YA_1) = 0 \\
f_5 &= X_3(XS_2 - XA_2) - XS_3(Y_2 - YA_2) = 0 \\
f_6 &= (X_3 + \delta_1)(XS_2 - XA_2) - XS'_3(X_3 - \delta_2 - YA_2) = 0
\end{aligned}
\qquad (5)
$$

Dans ces expressions δ_1 et δ_2 sont les distances le long des axes entre les origines des deux systèmes, sagittal et latéral (Fig 1)

Le système (5) a été résolu par la méthode des moindres carrés en utilisant la formule (3) et en imposant la condition de minimum

$$\frac{\partial F}{\partial X_i} = 0 \quad (i=1,3) \tag{6}$$

Pour les sources radioactives linéaires, est prévu un test de contrôle qui compare *la longueur calculée et celle qui fait partie des données d'entrée* Si l'erreur est supérieure à une certaine valeur choisie, le calcul s'arrête et une vérification de l'exactitude des coordonnées, lues sur les deux clichés pour cette source-là, s'impose

Pour certains types de sources, par exemple celles de radium, leur longueur réelle ne coincide pas avec leur longueur active Les caractéristiques de construction des sources étant connues, et uniquement pour le calcul de la dose, le programme utilise comme coordonnées des extrémités des sources celles qui correspondent à la longueur active, en les calculant à partir des coordonnées des extrémités de l'enveloppe, lues sur les deux films

Parmi les données d'entrée, outre la longueur réelle et la longueur active de chaque source, il est nécessaire de fournir les données relatives a la qualité et a l'épaisseur de l'enveloppe, le rayon intérieur de la substance radioactive pour tenir compte de l'autoabsorption, ainsi que le type et la quantité en mCi ou mg de l'isotope contenu dans la source

Identification des plans sur lesquels on désire effectuer le calcul des isodoses

C'est dans cette phase que nous nous sommes efforcés d'obtenir le maximum de résultats rendant possible l'étude détaillée de la distribution dans l'espace de la dose absorbée par le volume-cible, y compris les différents volumes surdosés et les volumes qui incluent le volume-cible et dont on doit tenir compte si l'on veut coordonner l'acte curiethérapeutique avec les autres modalités de traitement radiologique ou non

On a donc visé à laisser la plus grande liberté dans le choix qualitatif et quantitatif des plans, objet du calcul Le programme prévoit six variantes dans le choix qualitatif des plans de calcul

Le plan passe par trois points non alignés de l'espace, visibles dans tous les cas sur les deux radiographies orthogonales, tels que les repères radio opaques, anatomiques ou introduits artificiellement dans le malade, ou bien les extrémités de sources radioactives

Après avoir calculé par la méthode decrite au paragraphe précédent la position de ces trois points dans l'espace, le programme identifie le plan requis par les cosinus directeurs et la distance du repère fondamental

Le plan passe par deux points de l'espace, dont on peut lire les coordonnées sur les deux clichés, et est parallele à une direction donnée et non alignée avec ces derniers par exemple un axe cartésien du système fondamental de référence ou bien une direction quelconque

Le plan passe par un point de l'espace et est parallèle à deux directions choisies et bien distinctes

En suivant ces deux dernières voies de définition du plan dans lequel on fait le calcul, il est possible bien sûr d'identifier aisément des plans parallèles ou orthogonaux aux plans des films, comme l'ont proposé certains auteurs (ROSENWALD et coll 1970)

On definit les cosinus directeurs du plan et sa distance au repère absolu

Cette modalité d'identification est particulièrement utile pour effectuer le calcul dans des plans orientés d'une façon simple dans l'espace C'est le cas, par exemple, des plans des axes cartésiens du système de référence ou bien des plans dont les normales sont les bissectrices des axes étant tous situes à une distance donnee du repère fondamental de référence

Cette méthode est particulièrement appropriée à l'étude préalable des différentes dispositions géométriques des sources Toutefois, son utilité est évidente puisqu'elle permet, dans tous les cas, de definir les plans de calcul

Le plan est parallele à celui qui le précède et en est distant d'une certaine longueur Ce type de definition peut s'averer utile si l'on désire « couper en tranches » la zone etudiee par des plans paralleles entre eux et peut permettre l identification plus précise du volume traité avec une certaine dose

La définition du plan est donnée et découle d'études préliminaires ayant permis d'individualiser un plan caracteristique pour l'évaluation d'une certaine technique ou bien d'un certain aspect dosimétrique de celle-ci Nous avons prévu pour les applications gynécologiques, le calcul des isodoses dans un plan frontal, oblique, qui passe par la direction idéale des vaisseaux lymphatiques pelviens (DURRANCE et coll 1968).

En général, il faut indiquer les dimensions et l'agrandissement de la zone à explorer dans chaque plan requis Si l'agrandissement est unitaire, le diagramme des isodoses est imprimé en vraie grandeur, dans le cas contraire l'impression est effectuée dans les dimensions correspondant à l'agrandissement requis

Organisation du calcul en vue d'obtenir une représentation des résultats sous forme de diagrammes d'isodoses

Organisation generale du calcul

On prend comme centre pour le calcul le barycentre radioactif des sources radioactives, de façon que le diagramme des isodoses soit centré sur la zone la

plus intéressante du plan choisi, c'est à dire celle ou se projettent les sources Il est toutefois possible d'effectuer le calcul pour une partie du plan, differente et *éloignée de la projection* du barycentre d'une certaine distance et selon une certaine direction, ce qui est souvent utile pour évaluer la distribution de la dose au niveau de certaines structures ou régions anatomiques, éloignées du dispositif d'irradiation (chaînes lymphatiques, organes ou structures sensibles, etc)

Sur les plans choisis et identifiés durant la phase précédente, on calcule les projections des repères de référence, fondamental et accessoires, du barycentre radioactif et des extrémités des sources radioactives

Sur le plan est individualisé un nouveau système d'axes orthogonaux a deux dimensions, ayant *pour origine le barycentre du calcul,* systeme *dans lequel on* calcule les coordonnés des projections des repères de référence et des extremites des sources en vue de pouvoir les mettre en evidence lors de l impression du diagramme

Dans le système ainsi défini, on crée une grille de calcul dont les mailles sont d'autant plus serrées que l'agrandissement requis est plus grand et qui couvre la zone que l'on désire explorer Le détail du calcul est ainsi toujours proportionné à la demande tandis que les temps de travail ne dépassent jamais les besoins

Impression des diagrammes d'isodoses

Pour la représentation de la distribution dans l'espace de la dose absorbée on peut avoir recours à l'impression de tableaux à double entrée donnant la valeur de la dose calculée aux points d'intersection de la grille de calcul Ces nombres convenablement reliés par des lignes continues permettent de mettre en *évidence* l'allure des isodoses Ce type de representation n'est pas tres précis et ne visualise pas immédiatement, sur le plan clinique, les volumes de traitement

On peut obtenir un résultat beaucoup plus précis et facilement comprehensible en faisant dessiner les diagrammes par un traceur de courbes *Cette solution* a évidemment le desavantage d'un coût plus élevé et d'une augmentation des temps d'exécution Malgré les avantages esthetiques certains de cette solution, nous avons, par conséquent, preferé utiliser directement l'imprimante de l'ordinateur pour l'exécution graphique des diagrammes

On a suivi la methode qui consiste a faire imprimer alternativement des lettres par ordre alphabétique croissant et des espaces blancs, obtenant ainsi une visualisation appropriée des *isodoses calculées* (Fig 6) *Dans ce type de représentation* graphique des résultats, il existe une limite due aux dimensions finies du caractere de l imprimante Ce n'est, toutefois, qu'une limite apparente en effet, si on le juge nécessaire, il est possible d'augmenter a son gré le detail en augmentant l'echelle et en explorant des zones plus etroites du plan étudie

Les dimensions réelles des diagrammes sont conditionnées par les dimensions finies dans le sens de la largeur des feuille de l'imprimante (en moyenne 35 cm), tout à fait suffisantes d'ailleurs pour couvrir les distances en jeu

La valeur et le nombre des isodoses calculés et dessinées sont choisis à volonté et fixés dans les données d'entrée. Il est possible, en outre, d'obtenir le calcul de la dose absorbée en des points particulièrement intéressants préétablis et fixés ou bien calculés à partir de leurs coordonnées lues sur les deux films

Sur le diagramme des résultats ainsi organisés et imprimés, sont visualisées les projections des repères de référence et des extrémités des sources de façon à faciliter la reconnaissance de la géométrie du traitement et à permettre, en définitive, l application clinique des résultats

Calcul de la dose

Le programme prévoit le calcul de l'intensité horaire de la dose absorbée en tenant compte, au besoin, de la décroissance du radioisotope employé.

Les operations prevues sont possibles pour les sources ponctuelles ou linéaires, contenant les isotopes radioactifs les plus couramment employés et curiethérapie, sous forme de sels ou d'alliages, dans tous les cas dispersés dans l'espace et en différentes combinaisons qualitatives et quantitatives

On tient compte de la filtration due à l'enveloppe extérieure des sources, de l autoabsorption et de l'att[illegible]

m[illegible]

ve[illegible] proportionnel à leur rayon de courbure

Dans l elaboration du modèle mathematique pour le calcul de la dose, nous avons recherché la précision la plus elevée et compatible avec des temps de calcul raisonnables Ceux-ci sont relativement bas et de l'ordre de 60 secondes en utilisant l'ordinateur UNIVAC 1108, lorqu'on effectue le calcul pour deux sources radioactives, en quatre plans et pour 5 000 points par plan

Sources linéaires

Considerons une aiguille radioactive contenant, par exemple, du sel de radium, semblable à celle de la figure 4, et calculons l intensité de dose en un point quelconque On pourra écrire

$$I_p = \frac{G}{L} \Gamma c \int_{X_1}^{X_2} \frac{\varphi_1 \varphi_2}{r^2} dx \qquad (7)$$

Dans cette formule, G est la quantité d'isotope, L la longueur active, Γ est la constante spécifique d'exposition gamma du radioélément, c est le facteur de conversion des roentgen en rad, φ_1 et φ_2 sont des fonctions qui expriment l'atténuation de la radiation respectivement dans l'enveloppe métallique extérieure de la source et dans le milieu

En particulier φ_1 est à priori fonction de l'épaisseur effective de l'écran d, de l'angle ϑ et du coefficient d'absorption du matériau μ_m, tandis que φ_2 depend a priori, non seulement de ϑ et d, mais aussi de la distance y du point à l'axe de l'aiguille et du coefficient d'absorption dans les tissus μ_t

Si l'on considere comme négligeable l'atténuation de la radiation dans l écran et dans les tissus, c'est à dire si l'on pose φ_1 et $\varphi_2 = 1$, l'expression (7), en se référant à la figure 3, devient ·

$$I_p = \frac{G}{L}\frac{\Gamma c}{\Upsilon}\int_{\varphi_1}^{\varphi_2} d\vartheta = \frac{G}{L}\frac{\Gamma c}{\Upsilon}\left(\vartheta_2 - \vartheta_1\right) \tag{8}$$

Cette approximation est toutefois très grossière et peut entraîner des erreurs en certains cas très élevées et supérieures à 20—30 % Nous avons, par conséquent, jugé opportun d'insérer dans le calcul des expressions qui tiennent compte, d'une façon adéquate, de l'atténuation de la radiation dans l'enveloppe extérieure des sources et dans les tissus

Atténuation dans la gaine metallique Si l'on suppose la loi de Beer valable, l'atténuation de la radiation dans l'écran métallique peut être exprimée comme suit

$$\varphi_1 = e^{-\frac{\mu_m d}{\cos\vartheta}} \tag{9}$$

Dans cette expression, μ_m est le coefficient d'atténuation linéaire constant pour le matériau considéré et pour l'énergie moyenne des γ de l'isotope contenu dans la source

Même si l'on admet que les γ émis sont monoénergétiques, on a toutefois constaté qu'à cause de la radiation secondaire, la loi de Beer, rigoureusement valable pour les rayons γ fins et bien collimatés, se révèle insuffisante Si l'on veut conserver l'expression (9), il est nécessaire d utiliser un coefficient d'atténuation μ_m qui ne soit plus constant, mais qui varie en fonction de l'épaisseur traversée

$$\mu_m = f\left(\frac{d}{\cos\vartheta}\right) \tag{10}$$

Cette fonction decroît lorsque l épaisseur augmente étant donné que la contribution de la radiation secondaire croît (YOUNG & BATHO 1964)

Si l'on néglige l'atténuation dans les tissus, la dose au point P quelconque peut être alors calculée suivant l'expression :

$$I_p = \frac{G}{L}\frac{\Gamma c}{Y}\int_{\vartheta_1}^{\vartheta_2} e^{-\frac{\mu_m d}{\cos\vartheta}}\, d\vartheta = \frac{G}{L}\frac{\Gamma c}{Y}\left(U_1(\vartheta_2) - U_1(\vartheta_1)\right) \qquad (11)$$

Les intégrales $U_1(\vartheta)$, dans le cas où μ_m est constant, sont des intégrales de Sievert (1921, 1922) et peuvent être résolues avantageusement en intégrant par série

$$U_1(\vartheta) = \int_0^{\vartheta_2} e^{-\frac{\mu_m d}{\cos\vartheta}}\, d\vartheta = \vartheta - \mu_m d I_1(\vartheta) + \frac{(\mu_m d)^2}{2!} I_2(\vartheta) - \frac{(\mu_m d)^3}{3!} I_3(\vartheta) + \dots \qquad (12)$$

ou

$$I_1(\vartheta) = ln\left(\frac{1}{\cos\vartheta} + tg\vartheta\right)$$
$$I_2(\vartheta) = tg\vartheta \qquad (13)$$
$$I_n(\vartheta) = \frac{1}{n-1}\left[\left(\frac{1}{\cos\vartheta}\right)^{n-2} tg\vartheta - (n-2) I_{n-2}(\vartheta)\right]$$

Si μ_m est variable, il faut effectuer une intégration numérique, par exemple, par la méthode des intervalles finis. Dans ce dernier cas, le calcul est sensiblement plus long. On a cependant constaté qu'en utilisant une valeur appropriée et constante du coefficient d'atténuation (pour le radium $\mu_m = 1{,}52\ cm^{-1}$) les erreurs sont très limitées et de l'ordre de 2 %.

On a donc supposé la valeur $\mu_m = 1{,}52\ cm^{-1}$ pour le coefficient d'atténuation linéaire de la radiation γ du radium dans le platine. Pour les autres isotopes considérés, on a également supposé des valeurs de μ_m constantes et tirées de la littérature.

Dans l'expression (9) et dans les expressions suivantes, apparaît l'épaisseur effective d de l'écran. Cette valeur tient compte à la fois de l'autoabsorption dans le sel ou dans l'alliage de l'isotope et du fait qu'à cause du diamètre intérieur fini du matériel, certains rayons gamma traversent une épaisseur plus élevée que celle qui est mesurée radialement. Pour le calcul de l'épaisseur effective d, on a utilisé la formule proposée par Young & Batho (1964).

Atténuation dans les tissus. Au début, l'on jugeait que l'atté[illegible]

[illegible] mesures effectuées sur des modèles de grandes dimensions (Griffith 1933). Dans ces conditions, en effet, la radiation gamma secon-

daire diffusée compense presque complètement le phénomène d'absorption pour des distances aux sources peu élevées.

Les mesures expérimentales les plus récentes, ont permis de constater, au contraire, que pour des points même proches des sources, l'erreur que l'on commet en négligeant l'atténuation dans les tissus peut être élevée et supérieure à 20 %.

Si l'on prend comme mesure expérimentale de l'atténuation le rapport

$$\frac{\text{exposition dans l'eau}}{\text{exposition dans l'air}}$$

ce rapport coïncide avec le facteur de correction φ_2 dans le cas où l'on jugerait négligeable, comme en fait on a pu le constater expérimentalement, l'atténuation dans l'air. Or ce rapport n'est jamais unitaire pour n'importe quelle distance, même brève, à la source et, en général, il diminue quand celle-ci augmente.

Nous avons, par conséquent, jugé opportun d'insérer dans le calcul une expression tenant compte de l'atténuation de la radiation dans les tissus.

Nombreuses sont les expressions publiées, qui interprètent les données expérimentales et qui expriment, sous une forme généralement empirique, le lien entre la fonction d'atténuation φ_2 et la distance r aux sources, pour le radium. A titre de confrontation, nous reportons ci-après quelques-unes de ces expressions.

(BATHO & YOUNG 1964) $\varphi_2 = 1 - 0{,}0074\, r^{3,2}$ (14)

(SMOCOVITIS et coll. 1967) $\varphi_{2I} = e^{-0{,}01101\,0{,}00038 r\, r}$ (15)

(SMOCOVITIS et coll. 1967) $\varphi_{2II} = 1{,}0 - 0{,}010\, r^{1,25}$ (16)

(MEISBERGER & SHALEK 1965) $\varphi_2 = 1{,}0005 - 4{,}423 \times 10^{-3}\, r - 1{,}707 \times 10^{-3}\, r^2 + 7{,}448 \times 10^{-5}\, r^3$ (17)

Celles-ci donnent à peu près les mêmes résultats pour des valeurs de r comprises entre 0 et 10 cm, correspondant au champ d'expérimentation. Ces derniers divergent, notablement, pour des distances r plus élevées.

Sur le Tableau 1 ont été indiqués, pour différentes valeurs de r, les résultats obtenus avec les expressions mentionnées ci-dessus et, à titre de comparaison, plusieurs données expérimentales. On peut constater que l'expression proposée par MEISBERGER & SHALEK se présente comme assez satisfaisante non seulement pour $0 < r < 10$ cm, mais aussi pour des valeurs de r plus élevées. En outre, elle se présente sous une forme (développement en série de puissance) qui la rend particulièrement attrayante du point de vue du calcul par ordinateur.

Pour les autres isotopes considérés, nous avons également utilisé, afin d'exprimer l'atténuation de la radiation dans les tissus, l'expression de MEISBERGER & SHALEK (Tableau 2) en relevant, dans ce cas aussi, un accord convenable entre les données expérimentales et celles qui sont calculées (Tableau 3).

Tableau 1

Coefficients d'atténuation dans les tissus pour le radium 226. Comparaison entre valeurs expérimentales et valeurs théoriques suivant plusieurs auteurs

Distance en cm	Valeurs théoriques				Valeurs expérimentales					
	BATHO et coll (1964)	MEISBERGER et coll (1965)	SHOCOVITIS et coll (1967) (I)	SHOCOVITIS et coll (1967) (II)	WOOTTON et coll (1954)	TER POGOSSIAN et coll (1952)	VAN DILLA et coll (1952)	PONNUNNI KARTHA et coll (1966)	MEREDITH et coll (1966)	SHOCOVITIS et coll (1967)
0.0	1.00000	1.00500	1.00000	1.00000						
1.0	0.99260	0.99894	0.98821	0.99000						0.988
2.0	0.97907	0.98992	0.97488	0.97622	0.97	0.96		0.98	0.986	0.975
3.0	0.96155	0.97838	0.96008	0.96052					0.974	0.96
4.0	0.94080	0.96476	0.94388	0.94343	0.94	0.94	0.98	0.95	0.953	0.945
5.0	0.91727	0.94952	0.92635	0.92523					0.950	0.928
6.0	0.89124	0.93310	0.90759	0.90609	0.88	0.92	0.94	0.90	0.930	0.908
7.0	0.86295	0.91594	0.88768	0.88614						0.888
8.0	0.83256	0.89850	0.86672	0.86546	0.82	0.89	0.90	0.88	0.890	0.866
9.0	0.80020	0.88122	0.84480	0.84412						0.845
10.0	0.76599	0.86455	0.82201	0.82217	0.77	0.85	0.87	0.84	0.842	0.823
11.0	0.73003	0.84893	0.79847	0.79967						
12.0	0.69239	0.83482	0.77427	0.77665		0.80	0.82	0.79		
13.0	0.65315	0.82265	0.74951	0.75315						
14.0	0.61236	0.81288	0.72429	0.72919		0.76	0.77	0.74		

Tableau 2

Atténuation dans les tissus pour différents isotopes, suivant MEISBERGER & SHALEK

$\varphi_r = A + B \times r + C \times r^2 + D \times r^3$

Isotopes	A	B	C	D
^{60}Co	9.94[illegible]3 × 10[illegible]	−5.318 × 10[illegible]	−2.610 × 10[illegible]	1.327 × 10[illegible]
[illegible] Cs	1.009 × 10	−9.015 × 10[illegible]	−3.459 × 10[illegible]	−2.817 × 10[illegible]
[illegible] Au	1.0306 × 10	−8.134 × 10[illegible]	1.111 × 10[illegible]	−1.597 × 10[illegible]
[illegible] Ir	1.0128 10	5.019 × 10[illegible]	−1.178 × 10[illegible]	−2.008 × 10[illegible]

Tableau 3

Coefficients d'atténuation dans les tissues pour différent isotopes Comparaison entre valeurs expérimentales et valeurs théoriques, selon plusieurs auteurs

Distance en cm	Valeurs théoriques				Valeurs expérimentales							
	^{60}Co	^{137}Cs	^{198}Au	^{192}Ir	^{60}Co				^{137}Cs		^{198}Au	^{192}Ir
	Meisberger & Shalek (1965)				Van Dilla et coll (1952)	Wootton et coll (1954)	Ponnunni et coll (1966)	Meredith et coll (1966)	Ponnunni et coll (1966)	Meredith et coll (1966)	Meredith et coll (1966)	
0,0	0,99423	1,00910	1,03060	1,01280								
1,0	0,98643	0,99971	1,02342	1,01662								
2,0	0,97422	0,98946	1,01750	1,01797		0,98	0,98	0,990	0,95	1,00	1,014	0,985
3,0	0,95837	0,97818	1,01189	1,01671				0,976		1,00	1,01	1,008
4,0	0,93969	0,96570	1,00562	1,01274	0,97	0,93	0,95	0,960	0,93	0,99	1,00	0,99
5,0	0,91898	0,95186	0,99774	1,00593				0,938		0,985	0,983	0,976
6,0	0,89703	0,93647	0,98730	0,99617	0,93	0,87	0,89	0,915	0,90	0,957	0,982	0,980
7,0	0,87463	0,91938	0,97332	0,98332								
8,0	0,85259	0,90042	0,95487	0,96728	0,90	0,82	0,86	0,876	0,84	0,907	0,910	0,940
9,0	0,83170	0,87941	0,93096	0,94791								
10,0	0,81275	0,85619	0,90066	0,92511	0,86	0,78	0,85	0,838	0,81	0,862	0,873	0,895
11,0	0,79655	0,83059	0,86300	0,89874								
12,0	0,78388	0,80243	0,81701	0,86870	0,82		0,81		0,77			
13,0	0,77555	0,77156	0,76176	0,83485								
14,0	0,77235	0,73780	0,69626	0,79708	0,77		0,77		0,72			

Calcul de l'intensité de la dose. En introduisant les expressions obtenues de φ_1 et φ_2 dans l'expression (7), on obtient

$$I_p = \frac{G}{L}\frac{\Gamma c}{\Upsilon}\int_{\vartheta_1}^{\vartheta_2} e^{-\frac{\mu m d}{\cos\vartheta}}\left[A + B\left(\frac{y-d}{\cos\vartheta}\right) + C\left(\frac{y-d}{\cos\vartheta}\right)^2 + D\left(\frac{y-d}{\cos\vartheta}\right)^3\right]d\vartheta =$$

$$= \frac{G}{L}\frac{\Gamma c}{\Upsilon}\Big(U(\vartheta_2) - U(\vartheta_1)\Big) \qquad (18)$$

Si l'on développe l'exponentielle en série de Mac Laurin en fonction de $\frac{1}{\cos\vartheta}$ en s'arrêtant au quatrième terme et en négligeant les termes d'intéraction d'ordre supérieur au quatrième, on peut écrire

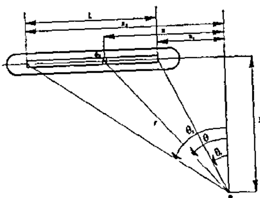


Fig 4 Schema d'une aiguille radioactive et de la geometrie du calcul de la dose en un point P quelconque

$$U(\vartheta) = AU_1(\vartheta) + (y-d)\,BU_2(\vartheta) + (y-d)^2CU_3(\vartheta) + (y-d)^3DU_4(\vartheta) \quad (19)$$

ou

$U_1(\vartheta)$ est l'intégrale de SIEVERT (12)

$$U_2(\vartheta) = I_1(\vartheta) - \mu_m dI_2(\vartheta) + \frac{(\mu_m d)^2}{2!} I_3(\vartheta) - \frac{(\mu_m d)^3}{3!} I_4(\vartheta)$$

$$U_3(\vartheta) = I_2(\vartheta) - \mu_m dI_3(\vartheta) + \frac{(\mu_m d)^2}{2!} I_4(\vartheta)$$

$$U_4(\vartheta) = I_3(\vartheta) - \mu_m dI_4(\vartheta)$$

et les intégrales I_1, I_2, I_3, I_4, ... sont calculées suivant (13). Malgré sa complexité apparente, la formule (19) se prête très bien au calcul de l'intensité de dose par ordinateur Elle est, grâce à des mesures opportunes, considérablement moins onéreuse du point de vue du temps de calcul qu'une intégration par la méthode des intervalles finis et, en outre, considerablement plus précise

La figure 5 représente les tracés de l intégrale $U(\vartheta)$ en fonction de ϑ avec $d = 0{,}05$ cm et $\mu_m = 1{,}52$ cm^{-1} et pour différentes valeurs de la distance y On peut constater combien ils s'écartent considérablement, surtout pour ϑ et y élevés, du cas $U(\vartheta) = \vartheta$ de la formule (8) où étaient négligées les atténuations dans la gaine metallique et dans les tissus En outre, pour des y élevés, on a des erreurs par rapport au cas $y = d$ ($\varphi_2 = 1$, intégrale de SIEVERT) de l'ordre, dans certains cas, de 10 % Ces erreurs sont considérablement amplifiées dans le calcul de la dose, celle-ci étant deduite de différences entre intégrales

La formule (19) ne converge pas pour des angles ϑ proches de $\frac{\pi}{2}$ ceci correspondant au fait que le développement en série de puissance de l'exponentielle fait défaut On a choisi comme domaine de validité de cette formule

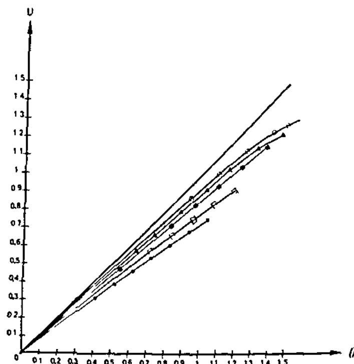


Fig 5 Tracés de l'intégrale $U(\vartheta)$ en fonction de ϑ, avec $d = 0{,}05$ cm et $\mu_m = 1{,}52$ cm^{-1}, pour différentes valeurs de y (○ 0,05 cm, ▲ 2,05 cm, ■ 4,05 cm, □ 8 05 cm, ● 16,05 cm)

$$\frac{\mu_m d}{\cos\vartheta} < 0{,}8$$

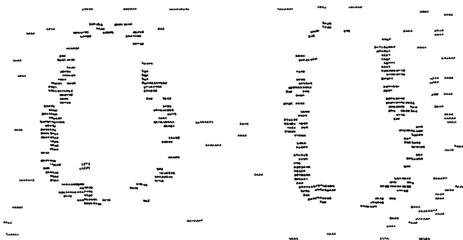
correspondant pour $\mu_m = 1{,}52$ cm^{-1}, $d = 0{,}05$ cm, à des valeurs de ϑ inférieures à environ 84 degrés

Pour des valeurs supérieures, on effectue la partie restante de l'intégration avec la méthode des intervalles finis et avec un nombre de pas limités D'autre part, il est opportun d'observer que, dans le calcul des isodoses pour un plan préétabli, la formule (19) ne converge plus pour les points situés uniquement tout près des sources

Sources ponctuelles

Pour les sources ponctuelles, toutes les considérations déjà faites en ce qui concerne l'atténuation de la radiation dans la gaine métallique et dans les tissus sont valables

Le calcul de l'intensité de dose est dans ce cas considérablement simplifié, étant donné qu'on ne doit plus effectuer d'intégrales





l axe de la sonde u erine c) Plan transversal a l axe de la sonde uterine au n eau des culs-de sac

$$I_p \quad \frac{G\Gamma c}{r^2} e^{-\mu m d}\left(1+B(r-d) + C(r-d)^2 + D(r-d)^3 \right) \qquad (20)$$

Le temps de calcul est inferieur au cas preceden m me

lu col de l utérus ent curietherapeutique pour cancer

L'analyse des diagrammes des isodoses requises (15, 22, 5, 45, 90, 135 rad/h), *permet l'étude du volume traité et* des volumes correspondants surdosés et irradiés.

Les isodoses sont visualisées à partir des limites des aires imprimées avec des lettres par ordre alphabétique croissant, la projection des extrémités des sources radioactives sur le plan de calcul est individualisée par des chiffres arabes par ordre progessif à partir de 0, tandis que la projection sur le plan des repères de référence est indiqué par des astérisques, à moins qu'il n'y ait superposition avec l'extrémité d'une source.

Le plan de traitment analysé prévoit : trois tubes de 10 mg de ^{226}Ra *de 22 mm* de long et de 16 mm de longueur active disposés en triangle dans les culs de sac avec le sommet dirigé vers la cloison vesico-vaginale et une sonde utérine composée de trois tubes de ^{226}Ra, de mêmes caractéristiques, mais avec une activite de 5 mg pour celui qui est situé au niveau du canal cervical et de 10 mg pour les tubes endo utérins (Fig. 6). Les plans de calcul sont respectivement : (1) plan frontal en a, (2) plan sagittal en b, (3) plan transversal au niveau des culs de sac en c.

Remerciements

Nous remercions pour son aide indispensable M. le Professeur Mario Dente, titulaire de la chaire de Principes de Genie Chimique à l'Institut de Chimie Industrielle, Politecnico, Milan.

RÉSUMÉ

Les auteurs relèvent la difficulte et l'importance d'une evaluation dosimétrique correcte en curiethérapie, et présentent les details d'un programme de calcul qu'ils ont élaboré permettant une solution adéquate du problème. Ce programme est d'un emploi simple et utile: il permet de satisfaire, au choix, deux exigences fondamentales: l'analyse des différentes dispositions géometriques des sources radio actives et l'évaluation, pour chaque cas particulier, des différents plans de traitement réalises sur le malade. Les auteurs présentent, en outre, quelques exemples de l'utilisation du programme.

SUMMARY

The authors discuss the difficulty as well as the importance of a correct dosimetric evaluation in curie therapy and give details of a means for its determination that they have evolved. The method is a simple and easy one and permits of two alternative fundamental requirements: the analysis of various geometric arrangements of the radiation sources and the assessment in each case of different ways of treating the patient. Several examples to illustrate the method are presented.

ZUSAMMENFASSUNG

Es wird auf die Schwierigkeiten und den Wert einer genauen Dosimetrie bei der Curie Therapie hingewiesen und eigene Methoden zur Erzielung genauerer Resultate empfohlen Die neue Methode ist einfach und rasch und befriedigt zwei Erfordernisse Erstens, eine Analyse der geometrischen Anordnung der Strahlenkorper und zweitens eine vergleichsweise Abschatzung verschiedener Bestrahlungsplane Einige Beispiele erkaren die Vorteile der Methode

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ACTA RADIOLOGICA

OFFICIAL ORGAN OF THE RADIOLOGICAL SOCIETIES OF
DENMARK FINLAND NORWAY AND SWEDEN

Vol 12 THERAPY PHYSICS BIOLOGY 1973
Fasc 4 August

METASTASES AND RECURRENCES IN NEPHROBLASTOMA

BERTA JEREB

Nephroblastoma produces metastases in the lungs, liver, regional lymph nodes, omentum, mesenterium, pleura, peritoneum, and less commonly the skeleton, brain and other organs (DARGEON 1960, GROSS & NEUHAUSER 1950, JEREB & EKLUND 1973, SCHWEISGUTH & BAMBERGER 1965, SULLIVAN & SUTOW 1969)

Metastases of a malignant growth constitute a grave condition with a poor prognosis More grounds for optimism, however, exist in nephroblastomas than in many other similar conditions for a wide range of cure rate has been reported (FARBER et coll 1960, KILMAN 1969, SCHWEISGUTH & BAMBERGER 1965, SCHWEISGUTH & SCHLIENGER 1967, SIROLA 1969, WEDEMYER et coll 1968, WHITE & KRIVIT 1962, VIETTEI et coll 1970)

A series of patients with metastases from nephroblastoma or recurrence was employed to analyse factors that might be considered to bear on (1) the frequency and site of these sequelae and (2) the outcome of their treatment

Material and Methods The 212 patients comprising the series were obtained from the 335 patients treated for nephroblastoma in Scandinavian hospitals between 1927 and 1967 and analysed in various respects previously The 9 patients dying at operation were not included in this series, and the 19 with bilateral

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Table 1

Patients with metastases during the three periods covered by the series

Period	No. of patients with metastases		
	At first treatment	Later	Total
1927–49	2	34	36
1950–59	18	63	81
1960–67	26	69	95
Total	46	166	212

nephroblastoma have been reported separately. A total of 46 patients had demonstrable metastases on admission, or these were detected at the first treatment. Of the remaining 261 patients 95 were cured with primary treatment and 166 developed metastases or recurrence after the first treatment had been completed (Table 1).

The staging of the disease, its application in retrospect, the methods of primary treatment and the retrospective calculation of the radiation dosage have been described elsewhere (JEREB & EKLUND 1973).

Since local recurrences and abdominal metastases of nephroblastoma were only rarely treated by surgery, the difficulty of distinguishing for the purpose of classification and reporting between local recurrences, residues, regional lymph node metastases and metastases in other abdominal structures arises. For the purpose of this investigation all these were therefore grouped together under the heading of abdominal recurrence. Pleural and mediastinal metastases could also not easily be differentiated from metastases in the lungs.

Fourteen of the 33 patients developing only pulmonary metastases after primary treatment had solitary and 19 multiple metastases, multiple metastases were located in both lungs. Because insufficient data were available the proportion could not be established for the patients treated before 1950. Metastases could not be located in retrospect in 18 patients (Table 2). The sexes were equally represented in the 46 patients with metastases at primary treatment and the 166 subsequently developing metastases or recurrence.

The age distribution in the 166 patients developing metastases or recurrence after primary treatment was as follows. Thirty-nine patients were under two, 71 were between two and four and 51 were between five and ten years old, five patients were over ten. The age distribution in the 46 patients with metastases at

Table 2

Site of metastases, 212 patients

Site	Patients with metastases			
	At first treatment	Later	Total	Per cent
Lungs only	26	67	93	44
Lungs and abdomen	12	32	44	20
Abdomen only	7	31	38	18
Lungs and skeleton	1	9	10	5
Skeleton only	0	6	6	3
Skeleton lungs and abdomen	0	3	3	1
Unknown		18	18	9
Total	46	166	212	100

the first treatment consisted of 7 patients under two years, 18 were between two and four, and 14 were between five and ten years, 7 patients were over ten

All the patients were followed up and in all of them the diagnosis was verified histologically

The methods of treatment varied—for example, from one patient to another and over the years In principle, the treatment was individualized The series was divided into three periods according to the year of admission 1927—49, 1950—59 and 1960—67 The second period corresponded roughly to the increasing use of high-voltage therapy equipment and more vigorous irradiation, and the third to the use of chemotherapy The first period embraced 36 patients with metastases or recurrence, the second 81, and in the third 95 patients (Table 1)

The methods for diagnosing pulmonary metastases remained essentially the same throughout the respective periods, and likewise the system of follow-up at the Scandinavian hospitals. On the other hand, a change in the attitude toward the treatment of patients with metastases or recurrence developed

No treatment for these was given to 25 of the 36 patients in the first period, 29 of the 81 patients in the second period or 22 of the 95 patients in the third period

Of the 46 patients with metastases on admission 7 received no treatment at all For the primary tumour 39 underwent nephrectomy, usually followed by irradiation, 17 of these received no treatment for their metastases and 22 patients did, 7 were given radiation therapy, chemotherapy or both

Table 3

Treatment of metastases or recurrence in 166 patients with metastases or recurrence detected after primary treatment

Method of treatment	No of patients
No treatment	52
Irradiation only	44
Surgery only	1
Chemotherapy only	6
Surgery + irradiation	5
Irradiation + chemotherapy	38
Surgery + chemotherapy	3
Surgery + irradiation + chemotherapy	4
Unknown	13

The combination of the various methods of treatment applied in 166 patients developing metastases or recurrence after primary treatment appears in Table 3 Actinomycin D was used in 48, cyclophosphamide in 13, vincristine sulphate in 3, nitrogen mustard in 2, sanamycin in one and Tiotepa in one patient

Intensive treatment was never withheld in any patient with pulmonary metastases solely on ground of poor general condition When such therapy was not given it was for the reason that metastases were present

The possibility of a correlation between the presence of metastases or recurrence and the following factors was examined (1) age of the patient at the first treatment, (2) stage of the disease at the time of the first treatment, (3) method of primary treatment, and (4) radiation doses at the primary treatment The results are presented in the tables

Only one factor at a time could be so analysed Several factors may, however, be correlated to each other and to the result of the treatment in such a way that correlation between one factor and the result might be attributed to other effects capable of influencing the result in the same direction For instance, patients in the third period were given higher radiation doses and often chemotherapy as well, the condition was also generally in a relatively early stage at the time of the first treatment (JEREB & EKLUND 1973) A multivariate 'tree' analysis was undertaken to evaluate the factors and their relative influence on the result simultaneously In this a series of independent variables (predictors) is examined in relation to a dependent (result) variable (SONQUIST & MORGAN 1970) This is done by the successive splitting of the original group into pairs of subgroups

Table 4

Time after primary treatment during which metastases were detected in 166 patients

Time after primary treatment months	No of patients	Per cent
< 3	67	40
3 – 5	48	29
6 11	27	16
12 – 14	10	6
15 23	4	2
24 35	2	1
≥36	3	2
Unknown	5	3

The independent variable that will produce a pair of subgroups differing as much as possible from each other with respect to the variable is chosen as a basis for the division After several successive divisions a tree' consisting of the subgroups is formed, the appearances of this being determined by the variables The division ends if the subgroup contains less than 10 patients, or two groups with respect to the dependent variable fail to fulfil the criteria of significance (EKLUND & GAVATIN 1972, JEREB & EKLUND 1973) However, a number of subgroups of the trees with differences not covering these criteria at the 5 per cent level are presented and are indicated in the figures by broken lines

The first step in the tree analysis was to determine the frequency of metastases in relation to the factors mentioned as well as to the calendar year on admission The result variables used were the frequency of extra abdominal metastases in one tree analysis and the frequency of abdominal metastases or recurrence in the second analysis

The relative importance of the various factors for the effect of the treatment of metastases or recurrence was then examined, the following factors being added as independent variables (1) method of treatment of metastases or recurrence (Table 3), (2) site of metastases or recurrence (Table 2), and (3) time elapsing between primary treatment and appearances of metastases or recurrence (Table 4)

The result variables in this analysis were the cure rate and the 2-year survival rate the latter being calculated from the day of the first treatment of metastases or recurrence The criterion adopted for a cure was the absence of any evidence of the disease three years or more after the last treatment All the patients classed as cured remained so until the end of this investigation in December 1970

Table 5

Frequency of metastases after primary treatment against age in 261 patients

Age	No of patients	Metastases	Per cent
< 2 years	97	42	43
> 2 years	164	124	76

Table 6

Frequency of metastases after primary treatment against stage in 261 patients

Stage at primary treatment	No of patients	Metastases	Per cent
Stage I	116	50	43
Stage II	57	36	63
Stage III	81	73	90
Not defined	7	7	

Table 7

Frequency of metastases after primary treatment against method of treatment in 261 patients

Primary treatment	No of patients	Metastases	Per cent
No treatment	3	3	
Irradiation only	10	10	
Surgery only	35	31	89
Surgery + irradiation	151	90	60
Surgery + irradiation + actinomycin D	62	32	52

Table 8

Frequency of metastases after primary treatment against irradiation dose at the primary treatment in 261 patients

Irradiation dose (rad)	No of patients	Metastases	Per cent
No irradiation	38	34	89
< 1000	19	14	(74)
1000 – 2000	55	31	56
2000 – 3000	79	48	61
> 3000	64	33	52
Dose unknown	6	6	

Table 9

Frequency of extra-abdominal metastases detected after primary treatment against age in 261 patients

Age	No of patients	Metastases	Per cent
< 2 years	97	17	18
≥ 2 years	164	65	39

Table 10

Frequency of extra abdominal metastases detected after primary treatment against stage in 261 patients

Stage at primary treatment	No of patients	Metastases	Per cent
Stage I	116	28	24
Stage II	57	19	33
Stage III	81	32	39
Not defined	7	3	

Table 11

Frequency of extra abdominal metastases after primary treatment against method of treatment in 261 patients

Primary treatment	No of patients	Metastases	Per cent
No treatment	3	3	
Irradiation only	10	3	
Surgery only	35	9	26
Surgery + irradiation	151	47	31
Surgery + irradiation + actinomycin D	62	20	32

Table 12

Frequency of extra abdominal metastases after primary treatment against irradiation dose at the primary treatment in 261 patients

Irradiation dose (rad)	No of patients	Metastases	Per cent
No irradiation	38	11	29
1000	19	5	(26)
1000 2000	55	14	25
2000 3000	79	27	34
≥ 3000	64	22	34
Dose unknown	6	3	

Table 13

Sites of metastases at autopsy in 41 patients with metastases or recurrence after primary treatment

Site	No of patients
Lungs	26
Liver	16
Omentum, mesentery	10 each
Pleura, mediastinum, local recurrence, retroperitoneal glands, peritoneum	6 each
Skeleton, pelvis	4 each
The other kidney, pancreas	3 each
Uterus	2
Myocardium, ovarium, vagina, appendix, peripheral lymph nodes	1 each

Results

The 46 patients who had metastases at the time of the first treatment died within 2 years, 90 per cent of them within one year, and 60 per cent within six months The variety of treatments used in these patients allowed only small groups to be formed, for this reason, and because of the short survival for the respective groups the analyses afforded no useful information

Of the 261 patients with clinical localized nephroblastoma at the time of the first treatment 166 (64 per cent) subsequently developed metastases or recurrences The first site detected was predominantly in the lungs (Table 2), in 85 per cent of the patients developing metastases or recurrence after the primary treatment these appeared within one year (Table 4) The frequency of metastases or recurrence was higher in the patients over 2 years of age on admission than in the younger ones (Table 5), the more advanced the local tumour (Table 6), in patients not receiving irradiation (Table 7), and probably also in the group in which the dose to the tumour bed was less than 1 000 rad (Table 8)

The frequency of extra-abdominal metastases was lower in patients under 2 years of age than in the more elderly (Table 9) and lower in the patients with a stage I tumour (Table 10) It was fairly constant, however, for the different methods of treatment (Table 11) and radiation dose groups (Table 12)

A total of 153 of the 166 patients developing metastases or recurrence after the primary treatment died Autopsies were performed in 41, the sites and frequency of metastases or recurrence appear in Table 13 In 8 of these patients no abdominal tumour was evident and in 8 of the other 33 the abdominal tumours were not diagnosed during life Local recurrences were present at autopsy in 6

Table 14

Thirteen patients with metastases of nephroblastoma Cured AMD = actinomycin D

Sex	Age (years)	Stage	Primary treatment		Metastases, site	Treatment for metastases		Without recurrence (years)
			Date	Method and dose		Date	Method and dose	
♀	6	II	1949 July	Nephrectomy + 2 500 rad	Lung solitary	1949 Nov	Irrad, local 2 500 rad	21
♀	3	II	1953 Jan	Nephrectomy + 2 500 rad	Lung multiple	1953 June	Irrad, local 2 500 rad multiple fields	16
♀	9	II	1958 June	Nephrectomy + 2 500 rad	Lung solitary	1958 July	Irrad, local 3 000 rad + AMD (single course)	10
					Abdomen (omentum)	1959 Jan	Surgery + irrad, local 3 000 rad	
♀	5	I	1959 May	300 rad + nephrectomy + 1 500 rad	Lung multiple	1960 May	Irrad, whole lung 1 000 rad + AMD (multiple courses)	10
♂	2	III	1959 Oct	Nephrectomy + 3 600 rad	Lung solitary	1960 April	Irrad local 3 000 rad + surgery	9
♀	2	I	1960 Sept	Nephrectomy + 2 800 rad	Lung solitary	1961 Oct	Irrad., local (dose unknown) + Sanamycin	9
	6	II	1961 Sept	Nephrectomy + 2 500 rad + AMD (single course)	Lung multiple	1962 Jan	Irrad, whole lung 2 000 rad + surgery + AMD (mult courses)	16
♂	3	I	1961 Oct	Nephrectomy + 2 000 rad	Lung multiple	1963 Jan	Surgery + AMD (single course)	7
♂	2	I	1963 July	Nephrectomy + 2 800 rad	Lung solitary	1964 May	Irrad local 3 700 rad	6
	2 12	I	1965 Febr	Nephrectomy + 2 600 rad + AMD (single course)	Lung multiple	1965 May	Irrad whole lung 600 rad + AMD (single course)	5
♀	5	II	1965 March	Nephrectomy + 2 800 rad	Lung solitary	1965 Nov	Irrad, local 4 000 rad	5
♂	7	I	1965 May	Nephrectomy + 2 200 rad + AMD (single course)	Abdomen (retrop glands)	1965 Oct	Surgery + AMD (single course)	5
♀	2	III	1967 Feb	Nephrectomy + 3 000 rad + AMD (single course)	Lung multiple	1967 July	Irrad., whole lung 1 500 rad + AMD (single course)	3

Table 13

Sites of metastases at autopsy in 41 patients with metastases or recurrence after primary treatment

Site	No of patients
Lungs	26
Liver	16
Omentum, mesentery	10 each
Pleura, mediastinum, local recurrence, retroperitoneal glands, peritoneum	6 each
Skeleton, pelvis	4 each
The other kidney, pancreas	3 each
Uterus	2
Myocardium, ovarium, vagina, appendix, peripheral lymph nodes	1 each

Results

The 46 patients who had metastases at the time of the first treatment died within 2 years, 90 per cent of them within one year, and 60 per cent within six months The variety of treatments used in these patients allowed only small groups to be formed, for this reason, and because of the short survival for the respective groups the analyses afforded no useful information

Of the 261 patients with clinical localized nephroblastoma at the time of the first treatment 166 (64 per cent) subsequently developed metastases or recurrences The first site detected was predominantly in the lungs (Table 2), in 85 per cent of the patients developing metastases or recurrence after the primary treatment these appeared within one year (Table 4) The frequency of metastases or recurrence was higher in the patients over 2 years of age on admission than in the younger ones (*Table* 5), *the* more advanced *the local* tumour (Table 6), in patients not receiving irradiation (Table 7), and probably also in the group in which the dose to the tumour bed was less than 1 000 rad (Table 8)

The frequency of extra-abdominal metastases was lower in patients under 2 years of age than in the more elderly (Table 9) and lower in the patients with a stage I tumour (Table 10) It was fairly constant, however, for the different methods of treatment (Table 11) and radiation dose groups (Table 12)

A total of 153 of the 166 patients developing metastases or recurrence after the primary treatment died Autopsies were performed in 41, the sites and frequency of metastases or recurrence appear in Table 13 In 8 of these patients no abdominal tumour was evident and in 8 of the other 33 the abdominal tumours were not diagnosed during life Local recurrences were present at autopsy in 6

Table 14

Thirteen patients with metastases of nephroblastoma Cured AMD = actinomycin D

Sex	Age (years)	Stage	Primary treatment Date	Primary treatment Method and dose	Metastases, site	Treatment for metastases Date	Treatment for metastases Method and dose	Without recurrence (years)
♀	6	II	1949 July	Nephrectomy + 2 500 rad	Lung solitary	1949 Nov	Irrad , local 2 500 rad	21
♀	3	II	1953 Jan	Nephrectomy + 2 500 rad	Lung multiple	1953 June	Irrad , local 2 500 rad, multiple fields	16
♀	9	II	1958 June	Nephrectomy + 2 500 rad	Lung solitary	1958 July	Irrad , local 3 000 rad + AMD (single course)	10
					Abdomen (omentum)	1959 Jan	Surgery + irrad , local 3 000 rad	
♀	5	I	1959 May	300 rad + nephrectomy + 1 500 rad	Lung multiple	1960 May	Irrad , whole lung 1 000 rad + AMD (multiple courses)	10
♂	2	III	1959 Oct	Nephrectomy + 3 600 rad	Lung solitary	1960 April	Irrad , local 3 000 rad + surgery	9
♀	2	I	1960 Sept	Nephrectomy + 2 800 rad	Lung solitary	1961 Oct	Irrad , local (dose unknown) + Sanamycin	9
♀	6	II	1961 Sept	Nephrectomy + 2 500 rad + AMD (single course)	Lung multiple	1962 Jan	Irrad whole lung 2 000 rad + surgery + AMD (mult courses)	16
♂	3	I	1961 Oct	Nephrectomy + 2 000 rad	Lung multiple	1963 Jan	Surgery + AMD (single course)	7
♂	2	I	1963 July	Nephrectomy + 2 800 rad	Lung solitary	1964 May	Irrad , local 3 700 rad	6
♀	2/12	I	1965 Febr	Nephrectomy + 2 600 rad + AMD (single course)	Lung multiple	1965 May	Irrad whole lung 600 rad + AMD (single course)	5
♀	5	II	1965 March	Nephrectomy + 2 800 rad	Lung solitary	1965 Nov	Irrad , local 4 000 rad	5
♂	7	I	1965 May	Nephrectomy + 2 200 rad + AMD (single course)	Abdomen (retrop glands)	1965 Oct	Surgery + AMD (single course)	5
♀	2	III	1967 Feb	Nephrectomy + 3 000 rad + AMD (single course)	Lung multiple	1967 July	Irrad , whole lung 1 500 rad + AMD (single course)	3

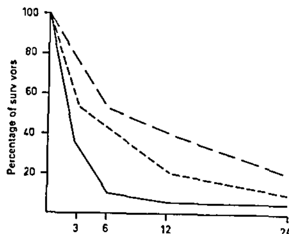


Fig 1

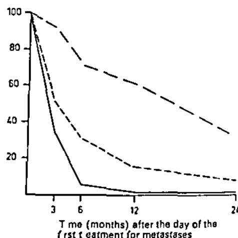


Fig 2

Fig 1 Survival of 166 patients with metastases or recurrence during the three periods —— 1927—49 ---- 1950—59 and — — 1960—67

Fig 2 Survival of 153 patients with metastases or recurrences distributed by method of treatment — no treatment ——— irradiation only, surgery only and chemotherapy only — — surgery + irradiation irradiation + chemotherapy, surgery + chemotherapy and surgery + irradiation + chemotherapy

patients and in only one had it been clinically diagnosed A growth in the other kidney in 3 patients was first detected at autopsy

Thirteen patients were recorded as cured Some relevant clinical data concerning these patients are presented in Table 14 Eleven of them had lung metastases only, 6 had solitary pulmonary metastases and in 5 they were multiple and bilateral Out of 51 patients given chemotherapy in addition to irradiation, surgical treatment, or both, 6 were cured Seven of 63 patients not given chemotherapy were cured

The survival rate for patients developing metastases or recurrence after primary treatment improved during the three periods (Fig 1) The survival curves indicate a higher 2-year survival rate in the patients treated by a combination of two methods (Fig 2) Two of the 4 patients treated by surgery, irradiation and chemotherapy were alive and without signs until the end of the investigation

A 'tree' in which abdominal recurrence was taken as the result variable is presented in Fig 3 The first split is made between the stages, stage I having a much lower frequency of abdominal recurrence than the other stages In the next division it would seem that the frequency of abdominal recurrence was lower

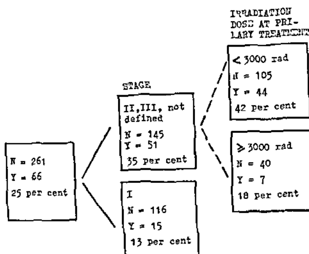


Fig 3 Frequency of abdominal metastases N – Number of patients Y – Number of patients with abdominal metastases 100 $\frac{Y}{N}$ per cent Coefficient of determination 9 per cent

for the higher radiation dose The difference between the subgroups obtained in this second division does not, however, fulfil the criteria of significance

A similar 'tree' for the frequency of extra-abdominal metastases appears in Fig 4 Thirty one per cent of the 261 patients developed such metastases, but no clinically known metastases within the abdomen. The original group is divided into two subgroups on the basis of the predictor age, the lowest frequency of extra-abdominal metastases occurring in patients under one year of age

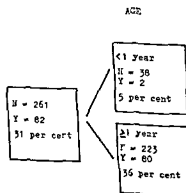


Fig 4 Frequency of extra abdominal metastases N = Number of patients Y = Number of patients with extra abdominal metastases 100 $\frac{Y}{N}$ per cent Coefficient of determination 5 per cent

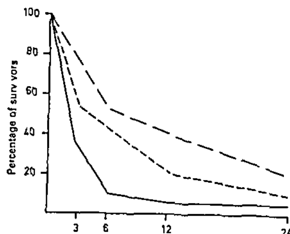


Fig 1

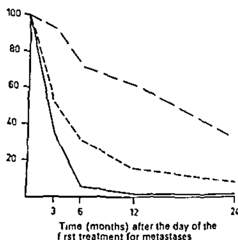


Fig 2

Fig 1 *Survival of 166 patients with metastases or recurrence during the three periods* —— 1927—49, - - 1950—59 and — — 1960—67

Fig 2 Survival of 153 patients with metastases or recurrences distributed by method of *treatment* — *no treatment* ——— *irradiation only*, surgery only and chemotherapy only — — surgery + irradiation, irradiation + chemotherapy, surgery + chemotherapy and surgery + irradiation + chemotherapy

patients and in only one had it been clinically diagnosed A growth in the other kidney in 3 patients was first detected at autopsy

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A 'tree' in which abdominal recurrence was taken as the result variable is presented in Fig 3 The first split is made between the stages, stage I having a much lower frequency of abdominal recurrence than the other stages In the next division it would seem that the frequency of abdominal recurrence was lower

The result of the analysis of the 2 year survival rate for the 166 patients devel oping metastases or recurrence after primary treatment are presented in Fig 5 Thirteen per cent of the patients survived for at least 2 years In this analysis the site of the metastases emerges as the main factor influencing the survival The 2 year survival for the group of patients with extra abdominal metastases was 23 against 4 per cent for those who also had abdominal metastases According to the second division the survival rate for the patients with extra abdominal metastases might also be influenced by the method of treatment

Of the 13 cured patients (Fig 6), those with only pulmonary metastases had a higher cure rate than those with metastases or recurrence at other sites The differences between these two subgroups and between the two subgroups ob tained by the second division in Fig 3 failed to meet the criteria of significance

Discussion and Conclusion

The prognosis for the patients with metastases at the time of the first treat ment was grave Few of them were treated for the metastases, and even then the therapy tended to be more restrictive, it was impossible to establish in retrospect whether the therapy was less active for the patients in poor condition

The rationale behind the classification of the metastases into abdominal re currence and extra abdominal metastases is that the former may represent short comings in the local treatment of the primary tumour, the latter on the other hand represent inability to prevent the spread of the tumour even though it may apparently have been successfully eradicated locally

The overall frequency of metastases and recurrences was correlated to the stage of the disease and the form of primary treatment This correlation has earlier been established (JEREB & EKLUND 1973) the percentage of cures being correlated to the form of primary treatment, this was not evident when the fre quencies of abdominal recurrences and extra abdominal metastases were analysed separately The two groups do, however, behave differently The only factor in fluencing the occurrence of extra abdominal metastases that could be established by the analysis was the age of the patients (Fig 4) Such metastases appeared not to be influenced by the stage of the primary tumour or to be preventable by more aggressive therapy (Tables 11, 12) Abdominal recurrence was dependent on the stage of the primary tumour and probably also on the radiation dose at the first treatment (Fig 3)

The coefficients of determination (JEREB & EKLUND 1973) in the analysis were low Only 5 per cent of the variation in the frequency of extra abdominal metastases and 9 per cent of that in the frequency of abdominal recurrence could be ascribed to the factors recorded (Figs 3, 4)

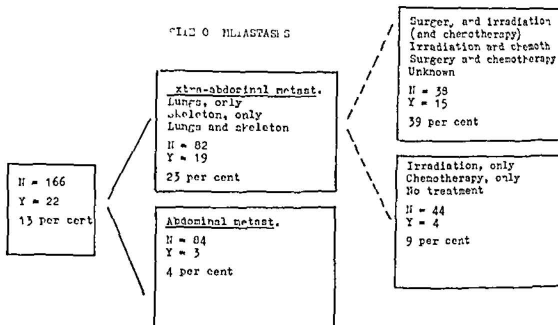


Fig 5 Two year survival rate in 166 nephroblastoma patients N = Number of patients Y = Number of survivors $100 \frac{Y}{N}$ per cent Coefficient of determination 15 per cent

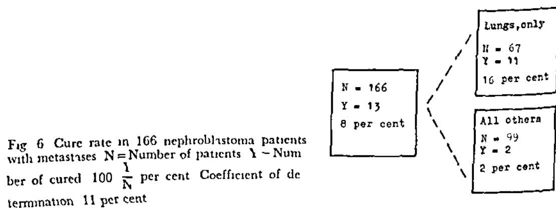


Fig 6 Cure rate in 166 nephroblastoma patients with metastases N = Number of patients Y = Number of cured $100 \frac{Y}{N}$ per cent Coefficient of determination 11 per cent

The result of the analysis of the 2-year survival rate for the 166 patients developing metastases or recurrence after primary treatment are presented in Fig 5 Thirteen per cent of the patients survived for at least 2 years In this analysis the site of the metastases emerges as the main factor influencing the survival The 2 year survival for the group of patients with extra-abdominal metastases was 23 against 4 per cent for those who also had abdominal metastases According to the second division the survival rate for the patients with extra-abdominal metastases might also be influenced by the method of treatment

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The results suggest that the occurrence of the metastases is governed in some measure by matters that were not recorded—for instance, the histologic type and the size of the tumour, its immunologic characteristics and the defence mechanisms of the host. The method of treatment for metastases or recurrence was correlated to the survival rate. This rate for the patients treated with a combination of two methods was better than that for those who received only irradiation, chemotherapy or surgery (Figs 2, 5). The number of patients subjected to combined surgery, irradiation and chemotherapy was too few to indicate whether this combination might produce still better results.

Patients with extra-abdominal metastases only had a higher 2-year survival rate, and those with only pulmonary metastases appeared to have had a higher cure rate than those with metastases to other organs as well (Figs 6, 7). A possible explanation of the more favourable outcome in patients with pulmonary metastases than in those with further metastases is that these were detected earlier. Pulmonary metastases unknown during life were not detected at autopsy. Five of the 33 patients with abdominal tumours post mortem had a local recurrence and 3 had a tumour in the other kidney which had not been detected during life.

A possible source of error is a selection of patients for the combined treatment; there might, for example, have been a tendency to choose patients with solitary metastases more often for surgery as well as to give larger radiation doses; moreover for those in poor general condition the therapy might have been more restrictive. It might thus be these selective factors, rather than the method of treatment, that are correlated to survival. The following facts, however, suggest that any such selection could hardly have been a decisive factor: (1) the cure rate was only slightly better, if at all, for the patients with solitary than for those with multiple bilateral pulmonary metastases; the ratio of solitary to multiple bilateral metastases was 6 : 5 for the cured patients and 14 : 19 for the 33 in whom this ratio could be established in retrospect; (2) the ratio of solitary to multiple bilateral metastases to the lungs was roughly the same in the second and third periods; the treatment of metastases and recurrences became more active in the third period and the results improved; (3) so far as could be ascertained, intensive treatment of metastases, including surgery and high radiation doses, was never withheld because of the patient's poor condition, but simply because metastases existed.

Acknowledgement

This investigation was supported by the Swedish Cancer Society and the Cancer Society of Stockholm.

SUMMARY

A retrospective statistical analysis was made of 212 patients with metastasizing nephro blastoma, treated in Scandinavian hospitals during 1927—67, forty six of these had metastases on admission The aim was to evaluate the factors influencing the frequency of me tastases and the outcome of their treatment Thirteen of the 166 patients in whom metas tases were detected after the primary treatment were cured The prognosis was more favourable when the metastases were confined to the lungs

ZUSAMMENFASSUNG

Es wurde eine retrospektive statistische Analyse von 212 Patienten mit metastasierendem Nephroblastom, die wahrend der Jahre 1927—1967 in skandinavischen Krankenhausern behandelt worden waren, vorgenommen, 46 der Falle hatten bei der Einweisung Metastasen Es war die Absicht die Faktoren festzustellen, die die Haufigkeit dieser Erkrankungsform und das Resultat deren Behandlung beeinflussen Dreizehn der 166 Patienten, bei denen Metastasen nach der primaren Behandlung festgestellt worden waren wurden geheilt Die Prognose war gunstiger, wenn es sich um Lungenmetastasen handelte

RÉSUMÉ

L auteur a fait une analyse statistique retrospective de 212 malades atteints de nephro blastome avec metastase traites dans les hôpitaux scandinaves entre 1927 et 1967, 46 de ces malades avaient des metastases au moment de leur admission Le but de cette recherche etait d apprecier les facteurs qui ont une influence sur la frequence de cette affection et sur le *resultat* de son traitement Treize des 166 malades chez qui les metastases ont ete decelees après le traitement primitif ont ete gueris Le pronostic est plus favorable quand les metastases sont limitees aux poumons

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RADIATION INDUCED PERICARDITIS IN HODGKIN'S DISEASE

R D MARKS JR, S K AGARWAL and W C CONSTABLE

The irradiation of Hodgkin's disease requires a sophisticated appreciation of normal tissue tolerance Attention was focused upon the heart and pericardium when STEWART et coll (1967) reported the first substantial series of patients with cardiac complications as a result of mediastinal irradiation They showed that the tolerance of the heart and pericardium is closely related to the volume irradiated and the dose delivered to the mediastinum The incidence of irreversible cardiac damage increases sharply as the dose exceeds 4 000 rad in four weeks in the customary regime of treating five days each week (STEWART & FAJARDO 1971) The authors have pointed out and demonstrated experimentally that cardiac fibrosis and constrictive pericarditis are both delayed manifestations of irradiation and that the symptoms of pericarditis usually do not appear until 6 to 30 months after irradiation

A review of the series of patients treated at the University of Virginia Hospital disclosed two cases of pericarditis, probably attributable to irradiation Certain features were present in these cases which bear directly on the dose required to produce pericarditis and the latent period before symptoms develop

Materials and Methods The records of all 235 patients with Hodgkin's disease from January 1956 through June 1971 were reviewed Of those registered, 137

Submitted for publication 25 April 1972

Table 1

Treatment techniques in mediastinal irradiation of 87 patients

Group	No of patients	Years	Radiation quality	Dose /time relationships	Daily treatment regime
A	16	1961—65	^{60}Co	3 000—3 500 rad, 20—25 fractions 5 days per week	All fields treated daily
B	18	1965—68	^{60}Co	4 000 rad, 20 fractions 5 days per week	Opposed fields treated on alternate days
C	14	1968—69	^{60}Co	3 500 rad, 15—18 fractions 5 days per week	All fields treated daily
D	19	1969—70	8 MV	3 500 rad, 15—18 fractions 5 days per week	Shaped mantle fields both fields treated daily
E	20	1970—71	8 MV	4 000 rad, 20 fractions in six weeks split course*	Shaped mantle fields both fields treated daily

* Two courses of 2 000 rad, in 10 days separated by two week rest interval

received radiation therapy, 115 of these received radical irradiation to all known areas of involvement The mediastinum was treated in 87 of these 115 patients and all received in excess of 3 000 rad The remaining 28 patients received radiation therapy to head and neck areas only or below the diaphragm The average age of these 115 patients was 33 years and there was an almost even split between the sexes

Treatment techniques The first Cobalt-60 teletherapy unit was installed in 1956 and provided a convenient date to commence this review All patients were treated by Cobalt-60 teletherapy or in the more recent years by 8 MV roentgen therapy Five periods in the evolution of treatment techniques can be identified (Table 1)

Results

Two patients developed cardiac complications which were attributed to irradiation Both of these patients demonstrated clinical, radiographic and ECG evidence of pericardial disease The first case occurred in treatment group B and presented as a classical case of pericardial effusion many months following treatment The second case occurred in group E and is considered to represent an example of acute radiation pericarditis in view of the extremely short interval between completion of the treatment and onset of symptoms



Fig 1 Case 1 a) Mediastinal adenopathy before radiation therapy b) Three months following irradiation with regression of adenopathy

Case I An 18 year-old white male with a painless swelling in the left lower cervical region of two months duration. No other symptoms were present Physical examination disclosed bilateral cervical and supraclavicular lymphadenopathy On chest films significant mediastinal adenopathy was apparent (Fig 1 a) but phlebography of the inferior vena cava and lymphangiography were negative All other laboratory values were within normal limits Employing the Rye Recommendations, he was staged II A on the basis of neck and mediastinal involvement and absence of symptoms Subsequent review has shown the histologic cell type to be nodular sclerosis. Three weeks after diagnosis, treatment was started to the nodal areas above the diaphraem employing a Cobalt-60 teletherapy unit Limitations of the machine and the size of the patient necessitated the use of three pairs of anterior and posterior fields directed to (1) the right neck and axilla, (2) the left neck and axilla (3) the mediastinum The mediastinal fields measured 10 cm × 15 cm and encompassed over 50 per cent of the cardiac silhouette 4 000 rad were delivered to the mid plane of the chest in an overall time of four weeks treating five days per week Only one field of each pair was treated daily The mediastinal interfield distance measured 27 cm and the maximum subcutaneous dose was calculated to be 5 100 rad Thus, that portion of the heart irradiated received from 4 000 to 5 100 rad during this course of treat ment In particular, it should be noted that the increments received by the anterior part of the heart on alternate days was 450 rad and this was repeated 10 times at a rate of 2 or 3 fractions per week

During treatment the patient experienced symptoms of moderate esophagitis which rapidly subsided on completion A chest film three months after treatment demonstrated complete regression of the hilar adenopathy but perihilar fibrosis was noted (Fig 1 b) The patient remained asymptomatic until 13 months following treatment when he developed moderate dyspnea on exertion and radiographic evidence of a pericardial effusion Based on his symptomatology, radiographic appearance and electrocardiographic findings, a diag nosis of pericarditis with pericardial effusion was made (Fig 2) He was placed on Prednisone no pericardial aspiration or surgery being considered necessary Over the ensuing



Fig 2 Case 1. Thirteen months after completion of irradiation with pericardial effusion

six months, he made an apparent uneventful recovery and his steroids were gradually discontinued The patient has been followed regularly and is now four years post treatment and remains well, free of Hodgkin's disease

Case 2 A 43-year-old white male with a one-year history of an asymptomatic left lower cervical mass There were no other symptoms present and his past history was unremarkable with no suggestion of cardiac disease Initial physical examination disclosed two small left lower cervical lymph nodes Biopsy of one of these was reported as Hodgkin's disease, mixed cellularity type The chest film was normal but a lymphangiogram was reported as equivocal The remainder of his laboratory and radiographic investigations was negative Staging laparotomy and splenectomy was performed with the findings of a positive para-aortic lymph node and a positive spleen Liver biopsy was negative He was staged *III A* and total nodal irradiation advised Radiation therapy was first directed to all disease above the diaphragm and he received 4 000 rad to the mid plane delivered by an 8 MV linear accelerator employing opposed mantle fields The mediastinal portion of the field averaged 10 cm in width and included approximately 60 per cent of the heart Treatment was administered over a six week period utilizing a split course as described in Table 1, group E He experienced nausea and symptoms of mild esophagitis and lost 4 5 kg in weight during this treatment Two weeks following completion of this phase of treatment, irradiation was started to all disease below the diaphragm employing an inverted Y-field Three weeks after completion of the mantle field, the patient began to complain of a vague anterior chest pain sometimes radiating to his shoulders There were no physical findings, and films of the chest were negative but the discomfort persisted requiring the intermittent use of Codeine for relief Because of a precipitous bone marrow depression when his platelet count fell from 280 000 to 40 000 per mm^3, this phase of treatment was protracted over a seven week period Eight weeks after completion of the mantle field irradiation, while still under treatment, the patient developed severe substernal chest pain requiring admission Several examiners noted a transient pericardial friction rub and serial electrocardiograms demonstrated low voltage and flat T waves suggestive of acute pericarditis The patient was placed on Prednisone and over a seven day-period his symptoms subsided and all clinical evidence of pericardial disease disappeared Over the following four months, the Prednisone was gradually discontinued and the patient appeared to make an uneventful recovery

Table 2

Nominal standard dose determinations in ret for cases 1 and 2

Position of NSD determination	Case 1 (Group B)			Case 2 (Group E)	
	NSD as treated (alternate fields daily, ^{60}Co)	Hypothetical NSD (both fields daily, ^{60}Co)	Hypothetical NSD (alternate fields daily, 8 MV)	NSD as treated (split course both fields daily 8 MV)	Hypothetical NSD (split course alternate fields daily, 8 MV)
Point of maximum dose	2150	1820	1812	1334	1580
1/4 interfield distance (heart dose)	1792	1518	1707	1317	1555
Mid plane (tumor dose)	1368	1368	1368	1295	1295

Dosage calculations Case 1 received a mid-plane dose of 4 000 rad in an [illegible]

[illegible] but in an overall time of 41 days delivered through opposed fields *treating* each field daily five days per week The 4 000 rad was delivered in two courses of 2 000 rad in 10 treatments, separated by a rest interval of 2 weeks *Although the* stated tumor dose is the same in both cases, the biologic effects are different due to the different treatment regimes In order to facilitate comparison, the nominal standard dose (NSD) was calculated in each case utilizing the formula (ELLIS 1969)

$$\text{Total dose} = \text{NSD} \times (\text{fractions})^{0.24} \times (\text{time in days})^{0.11}$$

The results of [illegible] inhomogeneity [illegible] maximum dosa[illegible] (subcutaneous dose) The 'heart dose' is intermediate between these values The reasons *for the inhomogeneity* are (1) treating one field on alternate days and (2) the large interfield distance (patient thickness) and (3) small percentage depth *dose For* comparison, the NSD has been calculated treating both fields daily The inhomogeneity is less but not eliminated reflecting the effect of patient thickness on depth dose

The results for case 2 show a lower mid plane NSD due to the prolonged overall treatment time consequent on the 'split', but this is not great The

homogeneity is very satisfactory reflecting the better depth dose achieved with 8 MV roentgen rays For comparison, the effect of treating one field on alternate days has been calculated The homogeneity is less satisfactory but not as poor as with cobalt-60 radiation

Discussion

Case 1 would appear to be acceptable as a case of radiation pericarditis with effusion as classically described At first sight, the dosage of radiation would seem to be within the tolerance level defined by STEWART & FAJARDO (1971) However, the biologic effect of treating one field on alternate days has been shown to be greater than treating all fields daily (WILSON & HALL 1971) The magnitude increases as the interfield distance increases The nominal standard dose in the region of the heart was, in fact, estimated to be 1792 ret, about 30 per cent greater than the mid-plane dose This is considerably higher than the 1 600 ret described by STEWART & FAJARDO as critical for the production of cardiac complications and compares with the 1 800 to 2 000 rad single dose he showed would produce an 87 per cent incidence of pericarditis in animals Table 2 shows that even with megavoltage equipment, treating one field on alternate days, especially in a stout patient, could raise the cardiac dosage to levels above tolerance Various methods such as shielding the heart, limiting total dosage to 3 500 rad or utilizing split course radiation therapy are all used to circumvent excessive irradiation to the heart All these methods have merit, but when high doses are utilized, treating all fields daily will offer an additional safety measure

Case 2 is a typical example of Hodgkin's disease involving the abdomen and left neck but not the mediastinum The dosage of 4 000 rad was delivered by 8 MV roentgen rays with a lower RBE than cobalt-60 and because of the split course, the biologic effect on the normal tissues was considered to be less The NSD throughout the treatment volume was rather low (Table 2) However, within three weeks of completing treatment, he was experiencing chest pain and subsequently a diagnosis of acute pericarditis was made Those cases in the series of STEWART et coll (1967) who developed pericarditis during or shortly after treatment all had Hodgkin's disease in the mediastinum In this case, there was no evidence of mediastinal or pericardial disease and so the latent period of three weeks seems unusually short The shortest latent period in STEWART's material when there was no mediastinal disease was six months There is some evidence that our patient was very sensitive to irradiation He lost a considerable amount of weight during treatment and developed an erythematous skin reaction with some areas of moist desquamation Symptoms of a moderate esophagitis were noted and he developed a marked and prolonged bone marrow depression

Although no other etiologic agents could be implicated in this case, that possibility must be kept in mind However in their absence, we accept this as an acute radiation pericarditis in an unusually sensitive individual

Two cases of pericarditis in 87 patients with a minimum of six months follow-up represents an incidence of 2 3 per cent It should be noted, however, that the incidence of cardiac complications was zero in the 47 patients in treatment groups A, C, and D who received a maximum of 3 500 rad This would concur with the observation reported by STEWART & FAJARDO that pericarditis is extremely rare when the mediastinal dose is limited to 3 500 rad in 3 5 weeks However, it should also be noted that all of these patients received irradiation to all fields daily

Conclusions

Any technique such as alternate field therapy which inadvertantly increases the biologic effects of irradiation should be avoided Sensitive individuals may exist who have a decreased normal cardiac tolerance to irradiation 3 500 rad administered from opposed fields with all fields treated daily has not produced cardiac complications in this series of patients

SUMMARY

In a series of 87 patients who received mediastinal irradiation for Hodgkin s disease two patients developed pericarditis which was attributed to the radiation therapy Both patients received 4 000 rad mid plane tumor dose one from a cobalt-60 unit to mantle fields treated on alternate days and the second by means of 8 MV roentgen rays utilizing a split course of treatment and treating both fields daily Employing nominal standard dose determinations case 1 was shown to have received a heart dose in excess of tolerance, the treatment of one field on alternate days contributing to the excessive dosage The heart dose in case 2 was within tolerance but acute pericarditis developed within six weeks of radiation therapy The possibility of individual sensitivity to irradiation is considered

ZUSAMMENFASSUNG

In einer Serie von 87 Patienten die eine Bestrahlung des Mediastinums wegen einer Hodgkin schen Erkrankung erhalten hatten entwickelten zwei Patienten eine Perikarditis die der Strahlentherapie zuzuschreiben war Beide Patienten erhielten eine Mittelplan Tumordosis von 4 000 rad bei dem einen wurden jeden zweiten Tag mit einer ^{60}Co Einheit Mantelfelder bestrahlt und der zweite wurde mit 8 MV Strahlen bestrahlt wobei eine Split course Behandlung mit täglicher Behandlung beider Felder vorgenommen wurde Bei Anwendung normaler Standarddosis Bestimmungen zeigte sich, dass der erste Fall eine über der Toleranzgrenze liegende Herzdosis erhalten hatte, wobei die Behandlung eines Feldes jeden zweiten Tag zu der zu hohen Dosis beigetragen hatte Die Herzdosis beim

zweiten Fall lag innerhalb der Toleranzgrenze, dennoch entwickelte sich eine akute Perikarditis innerhalb von 6 Wochen bei der Strahlentherapie. Die Möglichkeit einer individuellen Empfindlichkeit gegenüber Strahlung wird erwogen.

RÉSUMÉ

Sur une série de 87 malades ayant subi une irradiation médiastinale pour maladie de Hodgkin, 2 malades ont présenté une péricardite attribuée au traitement par les radiations. Ces deux malades avaient reçu 4 000 rad dans le plan moyen de la tumeur, l'un par cobalt thérapie sur des champs en mantelet traité un jour sur deux et le second par 8 MV des rayons de roentgen utilisant un traitement fractionné et traitant les deux champs chaque jour. La détermination de la dose standard nominale a montré que le cas 1 avait reçu une irradiation cardiaque dépassant la dose tolérable, le traitement d'un champs un jour sur deux contribue au dosage excessif. La dose au cœur dans le cas 2 était dans les limites de tolérance mais une péricardite aigue est apparue dans les six semaines qui ont suivi le traitement par les radiations. L'auteur envisage la possibilité d'une sensibilité individuelle à l'irradiation.

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RADIATION NECROSIS OF THE HUMERUS

A report of three cases

S SENGUPTA and K PRATHAP

Damage to underlying bone is one of the major complications of radiation therapy FAILLA (1921) and EWING (1926) were the first to point out such changes in bones of the jaw, and BAENSCH (1927) described fractures of the neck of the femur after radiation therapy to the pelvis, since then numerous authors have described such fractures, usually in middle aged women after irradiation of the pelvis for malignancy Radiation damage to other bones has also been described, e g the mandible (KANTHAK 1941), ribs (PAUL & POHLE 1942), pelvis (GRATZEK et coll 1945, STAMPFLI & KERR 1947), and spine (WHITEHOUSE & LAMPE 1953) The frequency of osteonecrosis of the femoral neck and pelvis are explained by the extensive use of radiation therapy in pelvic tumours Although this form of treatment is also much used in the treatment of carcinoma of the breast, it is surprising that no report of radiation necrosis of the humerus has appeared in the English literature KOLAR et coll (1967) described a case of erosion of the neck of the humerus some years after radiation therapy although neoplastic infiltration and infection could not be eliminated

Submitted for publication 13 June 1972



Fig 1 Case 1 Right shoulder Bone erosion in posterolateral aspect of head of humerus with collapse of the articular surface and generalised osteoporosis

Three cases of histologically proved radiation necrosis of the head of the humerus have been observed by the authors. Such changes are not unusual although they may me misinterpreted as being due to secondary deposits.

Case reports

Case 1 Female aged 50, Indian, with radical mastectomy fourteen years previously for carcinoma of the right breast This was followed immediately with a course of external radiation administered with a 200 kV source (FSD 40 cm, filter HVL 2 5 mm Cu), 120 applications in 24 sessions spread over a period of 32 days Total skin dose 4 000 R to right parasternal and 3 400 R to each of medial and lateral right chest fields with 2 850 R each to anterior and posterior right axillary and supraclavicular fields Each daily fraction was approximately 140 rad with an effective total tumour dose of about 3 350 rad

About three years later the patient had a hard lump in the right axilla A further dose of 3 600 R was given from the same source directly to the right axilla (field size 8 cm × 10 cm) with 18 treatments in 27 days, each application of 200 rad being administered in three minutes and four seconds

She was then well for about seven years when she noticed increasing pain and stiffness in the right shoulder No chest nor axillary recurrence nor any signs of distant metastases were present but there was marked tenderness of the humerus and the shoulder movements were much restricted Roentgenography An area of bone erosion of the posteromedial aspect of the humeral head and collapse of the overlying articular surface with mottled sclerosis around it was evident (Fig 1) The rest of the humerus and scapula were osteoporotic but the joint space was preserved



Fig 2 Case 1 Tissue removed from head of humerus Bone lamellae with empty lacunae denotes cell death Numerous thin walled blood vessels present in the surrounding fibrous tissue Mason trichome ×240

Histology of the excised area revealed that some of the cartilage and bone were replaced with granulation tissue (Fig 2) The debris consisted of irregular trabeculae of woven bone in which most of the lacunae were empty to indicate death of the osteocytes osteoblastic activity and osteoclasts were present only in one small area The marrow space was replaced by loose connective tissue in which many normal blood vessels and capillaries some spindle shaped cells and a few round cells were evident There was no sign of malignancy

Case 2 Female aged 70 Chinese underwent mastectomy for a variant of scirrhous carcinoma of the left breast in September 1962 this was followed by a course of deep roentgen therapy which was completed three months later With a 200 kV source (FSD 40 cm filter HVL 1 9 mm Cu) 70 applications in 26 days spread over 40 days were needed to give an effective total tumour dose of 4 520 R A skin dose of 4 500 R to the left parasternal field (15 cm×5 cm) and 5 250 R to left axillary and supraclavicular fields both anterior and posterior field sizes 10 cm×20 cm) were delivered the average daily fraction for each field being about 200 rad The patient was well until about seven years later when she started to have progressive swelling of the left upper limb with left brachial paresis The following year the axillary glands were not palpable and no recurrence was evident The left shoulder movements were restricted and painful and roentgen examination disclosed almost complete destruction of the articular surfaces with patchy sclerosis and widespread osteoporosis (Fig 3) The bony changes were consistent with radiation effects



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Fig 3 Case 2 Left shoulder Almost complete destruction of articular surface of the humerus with patchy sclerosis and widespread osteoporosis in the underlying bone

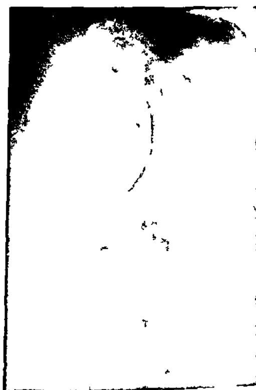


Fig 4 Case 3 Right shoulder Widespread osteoporosis and thinning of the shaft with fracture of the surgical neck Both humerus and scapula have patchy sclerosis

but metastases could not be eliminated On exploration, the bone debris appeared to be necrotic the soft tissues were vascular but no macroscopic malignant infiltration was evident

Biopsy of a specimen from the head of the humerus disclosed bone trabeculae with osteoid and dense connective tissue (Fig 5) Some of these appeared normal but localised areas of disorganisation of bone with absence of osteocytes from the associated lacunae were apparent The marrow space contained adipose tissue which was irregularly fibrotic Many blood vessels without changes of any significance were observed

Case 3 Female aged 50 Chinese had eight years previously undergone radical mastectomy for carcinoma of the right breast followed by irradiation (200 kV FSD 40 cm filter HVL 2 mm Cu) with 71 treatments at 24 sessions over a period of 35 days Skin dose 4 400 R to each anterior and posterior axilla (field sizes 10 cm × 15 cm) and 4 500 R to right parasternal area (field size 20 cm×5 cm) produced an effective total tumour dose of 4 515 rad Each daily fraction was 150 R for the axillary fields and 200 R for the parasternal field Seven years later the patient fell and sustained a pathologic fracture of the neck of the right humerus Ovariectomy was performed but failed to relieve the pain and the inability



F g 5 Ca e 2 T ssue from head of left humerus Degenerated bone lamellae w th abundant blood vessels and cap llaries n surround ng t ssue H & E×240



F g 6 Case 3 T ue from the s e of fracture Dead trabeculae w th bl od vessels n surround ng t ssues No mal gnant cells H & E×240

to move the right arm Roentgen examination eight months later demonstrated that the proximal two thirds of the humerus were grossly osteoporotic with patchy sclerosis and the surgical neck was fractured (Fig 4) The glenoid process of the scapula was also involved A diagnosis of radiation necrosis was confirmed by biopsy which revealed spicules of bone with empty lacunae and with many capillaries and small blood vessels (Fig 6) No evidence of malignant deposits The fracture was subsequently treated by internal fixation

Discussion

Irradiation is known to cause both cellular and vascular damage Their relative importance in the pathogenesis of radiation necrosis of bone is still a subject of debate and has been extensively discussed by RUBIN & CASARETT (1968) A striking feature of irradiation damage is osteoporosis, probably resulting from hyperaemia (STAMPFLI & KERR 1947, BONFOGLIO 1953). BAENSCH (1932) and TRUETA SEN (1942) observed the absence of osteoblasts in irradiated bone and concluded that increased vascularity was the cause of the osteoporosis which led to the fracture

On the other hand impairment of blood supply is known to result from irradiation EWING (1926, 1929) had originally reported that irradiation may produce thickening, fibrosis and obliteration of blood vessels Experimentally induced retardation of bone growth in the young occurs after the irradiation of immature bones from destruction of their blood supply (GRATZEK 1945) OKRAINETZ & BILLER (1939) and KOK (1953) have attributed the changes to damage leading to avascular necrosis

Fractures of the neck of the femur are notoriously difficult to heal and avascular necrosis is to be expected in one third of all traumatic fractures (WATSON JONES 1962) The fact that irradiation fractures of the neck of femur usually unite without avascular necrosis (BONFOGLIO 1953) suggests that there cannot be any material loss of circulation It is possible that because of the spotty distribution of the damage, the number of patent vessels observed in a given histologic slide may overestimate the number of unaffected vessels (RUBIN & CASARETT 1968) It is however likely that the gradual onset of changes allows time for the collateral circulation to be developed (BICKEL et coll 1961)

All the three cases now presented had no viable osteoblasts nor new bone formation together with the presence of normal capillaries and blood vessels This suggests that lack of normal dynamic osteoblastic activity is responsible for the thinning of the bone and the osteoporosis that leads to the pathologic fracture

Irradiation necrosis of the pelvis and femoral neck usually occurs within three years of the therapy (STEPHENSON & COHEN 1956, MURPHY 1967) Signs appeared seven to ten years after irradiation in the three cases now reported The humerus is not a weight bearing bone and pathologic fractures are probably

delayed, this is probably the reason for the late presentations The radiographic appearances of the changes in bone may simulate metastatic bone lesions and biopsy is always indicated Unlike a secondary malignant deposit, postirradiation bone necrosis of course carries a good prognosis

Acknowledgements

The authors are grateful to Prof J F Silva and S Dharmalingam for permission to *publish the case reports*

SUMMARY

Three cases of rad ation necrosis of the upper end of the humerus associated with the treatment of carcinoma of the breast are presented The changes in the affected bone were probably caused by primary cellular damage The appearances and differential diagnosis are d scussed

ZUSAMMENFASSUNG

Drei Falle einer Bestrahlungsnekrose des oberen Endes des Humerus im Zusammenhang *mit der* Behandlung eines Brustkarzinoms werden beschrieben Die Veranderungen des betroffenen Knochens waren wahrscheinlich durch einen primaren Zellschaden verursacht Das Erscheinungsbild und die Differentialdiagnose werden diskutiert

RÉSUMÉ

Les auteurs presentent trois cas de radio necrose de l extremite superieure de l humerus due au traitement du cancer du sein Les lésions dans l os atteint sont probablement causees par des les ons cellulaires primitives Les auteurs examinent les aspects radiologiques et le diagnostic differentiel

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COMMON ORIGIN FOR ALL NEUROENDOCRINE TUMORS

S L GAMMILL and R WEICHERT

Believ'. ; we have reasonably well established the carcinoid islet cell concept, we feel obligated to explore what we believe to be significant overlap between carcinoid islet cell tumors and the other neuroendocrine tumors The other neuroendocrine tumors, which are thought to originate embryologically from the neural crest and to be of ectodermal origin, include tumors of any of the glands that secrete peptide hormones (anterior pituitary, thyroid, parathyroid, carotid body, thymus, adrenal medulla) The ovaries, testes and adrenal cortex originate from the nephrogenic ridge, are thought to be of mesodermal origin,

mors may secrete gastrin, insulin, It is even more striking that what were formerly called carcinoid tumors have been associated with the production of glucagon, ACTH, MSH, catecholamines, kallikrein, and bradykinin (20, 24) What were formerly called islet cell tumors have been implicated in the production of glucagon ADH, catecholamines, ACTH, MSH, and secretin (5, 11, 24—27, 30) If we lump the carcinoid islet cell tumors together we may say that these tumors are potentially capable of secreting almost any of the peptide hormones Furthermore, chemodectomas have also been fo... creted

VMA

Submitted for publication 31 July 1972

Medullary thyroid carcinomas have been known to secrete ACTH and produce Cushing's syndrome. On bioassay of 2 of these tumors in patients with Cushing's syndrome, DONAHOWER et coll. found elevated ACTH levels in the tumor cell. One of these patients also had *pheochromocytomas in both adrenal medullas* (3).

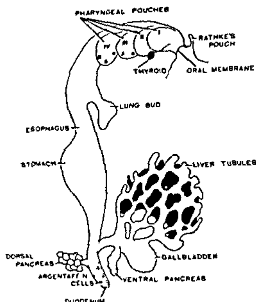
Werner's syndrome (multiple endocrine adenomatoses, MEA) is a well established entity. According to other authors, it occurs in as many as 25 per cent or more of patients with the Zollinger-Ellison syndrome (19, 29, 31). *Eighteen per cent of the patients with carcinoid islet cell tumors that we presented* in an earlier paper (7) had other neuroendocrine tumors and thus the MEA syndrome. There is also a familial form of this syndrome.

The most common variant of the MEA syndrome includes adenomas of the anterior pituitary, parathyroid and pancreatic islets. Carcinoids have also been associated with this syndrome relatively frequently (6, 10, 23, 24).

So grouping carcinoid and islet cell tumors together, we may say that the most common variant of the MEA syndrome is: tumors of the anterior pituitary gland, of the parathyroid gland, and carcinoid islet cell tumors. All of these tumors can be related to neural ectoderm and all are capable of producing peptide hormones. The second most common variant of the MEA syndrome is tumors of the thyroid, adrenal medulla and adrenal cortex. One of us (R. W.) has suggested that the association of the adrenal cortex to the tumors of the thyroid and adrenal medulla is probably not a true part of the MEA syndrome but is an incidental or secondary phenomenon (24). The medullary thyroid carcinoma-pheochromocytoma-neurofibromatosis syndrome is clearly of neural ectodermal origin and is also considered to be a variant of the MEA syndrome (24).

Chemodectomas have been found in association with thyroid carcinomas in several patients, and it was postulated that this association is possibly a neural ectodermal dysplasia related to the medullary thyroid carcinoma-pheochromocytoma-neurofibromatosis syndrome (1). Medullary thyroid carcinomas have also been associated with functioning parathyroid adenomas (4, 8, 13, 15, 16, 17, 18, 21, 24). The association of thyroid and parathyroid adenomas links the two main variants of the MEA syndrome previously mentioned and thus suggests that the entire MEA syndrome may be a dysplasia of neural ectoderm. The familial variant of this syndrome supports this hypothesis.

The following tumors have been associated with the production of serotonin: carcinoid islet cell tumors, acinar and ductal carcinomas of the pancreas, oat cell, squamous cell and adenocarcinomas of the lung, medullary carcinomas of the thyroid gland and neuroblastomas. Some of these tumors have also produced insulin, ACTH, and catecholamines (24—27). They seemed however to be linked by their production of a common hormone, serotonin. Therefore since



Diagrammatic representation of the distribution of argentaffin cells in the nervous system gastrointestinal tract and their derivatives The dots represent sites where argentaffin cells have been found and reported
● Parathyroid ▲ Thymus ■ Ultimobranchial body

serotonin production is associated with argentaffin cells, and since all these tumors secreted serotonin, it would seem logical to assume that they contained argentaffin cells or precursors to argentaffin cells, even though the tumors are not the same histologically All of these tumors can either be related to neutral ectoderm or arise where neuroectodermal cells are found

All of these facts point to a functional overlap among the neuroendocrine tumors that is more than casual and clearly shows that neuroendocrine tumors of different histologic types may secrete a common hormone (e g anterior pituitary tumors and medullary thyroid carcinomas may secrete ACTH) and neuroendocrine tumors of the same histologic type may secrete different hormones (carcinoid islet cell tumors may secrete insulin, gastrin, serotonin, kallikrein, catecholamines, etc) It has been suggested previously by one of us (R W) that all the neuroendocrine tumors arise from a single stem cell, the argentaffin cell (26), which originates in the neural crest embryologically These cells may be found normally in the central nervous system, stomach, gallbladder, pancreas and small bowel (Figure)

The histologic type of tumor that develops from this common stem cell has not been explained, and we have no good explanation, many factors are probably responsible That there is such a significant overlap in function among the neuroendocrine tumors and that there is a multiplicity of tumors in some patients

Medullary thyroid carcinomas have been known to secrete ACTH and produce Cushing's syndrome. On bioassay of 2 of these tumors, in patients with Cushing's syndrome, DONAHOWER et coll. found elevated ACTH levels in the tumor cells. One of these patients also had pheochromocytomas in both adrenal medullas (3).

Werner's syndrome (multiple endocrine adenomatoses, MEA) is a well established entity. According to other authors, it occurs in as many as 25 per cent or more of patients with the *Zollinger-Ellison* syndrome (19, 29, 31). Eighteen per cent of the patients with carcinoid islet cell tumors that we presented in an earlier paper (7) had other neuroendocrine tumors and thus the MEA syndrome. There is also a familial form of this syndrome.

The most common variant of the MEA syndrome includes adenomas of the *anterior pituitary, parathyroid and pancreatic islets*. '*Carcinoids*' have also been associated with this syndrome relatively frequently (6, 10, 23, 24).

So, grouping carcinoid and islet cell tumors together, we may say that the most common variant of the MEA syndrome is tumors of the anterior pituitary gland, of the parathyroid gland, and carcinoid-islet cell tumors. All of these tumors can be related to neural ectoderm and all are capable of producing peptide hormones. The second most common variant of the MEA syndrome is tumors of the thyroid, adrenal medulla and adrenal cortex. One of us (R. W.) *has suggested that the association of the adrenal cortex to the tumors* of the thyroid and adrenal medulla is probably not a true part of the MEA syndrome but is an incidental or secondary phenomenon (24). The medullary thyroid carcinoma-pheochromocytoma-neurofibromatosis syndrome is clearly of neural ectodermal origin and is also considered to be a variant of the MEA syndrome (24).

Chemodectomas have been found in association with thyroid carcinomas in several patients, and it was postulated that this association is possibly a neural ectodermal dysplasia *related to the medullary thyroid carcinoma-pheochromocytoma*-neurofibromatosis syndrome (1). Medullary thyroid carcinomas have also been associated with functioning parathyroid adenomas (4, 8, 13, 15, 16, 17, 18, 21, 24). The association of thyroid and parathyroid adenomas links the two main variants of the MEA syndrome previously mentioned and thus suggests that the entire MEA syndrome may be a dysplasia of neural ectoderm. The familial variant of this syndrome supports this hypothesis.

The following tumors have been associated with the production of serotonin: carcinoid-islet cell tumors, *acinar and ductal carcinomas* of the pancreas, oat cell, squamous cell and adenocarcinomas of the lung, medullary carcinomas of the thyroid gland and neuroblastomas. Some of these tumors have also produced insulin, ACTH, and catecholamines (24—27). They seemed *however* to be linked by their production of a common hormone, serotonin. Therefore, since

tumors aimed at providing a clearer understanding of them and their relationships to each other

ZUSAMMENFASSUNG

Die neuroendokrinen Tumoren, die embryologisch von der Neuralleiste herstammen, sezernieren Peptidhormone und bestehen aus Karzinoid Inselzell Tumoren, Tumoren des Hypophysenvorderlappens der Thyreoidea und Parathyreoidea, Chemodektomen, Phaeochromozytomen und anderen Tumoren, diese zeigen signifikante funktionelle Überschneidungen und sind enger miteinander verwandt als zuvor angenommen. Die Autoren haben ein neues Nomenklatursystem für diese Gruppe von Tumoren konstruiert, das ein besseres Verständnis dieser Tumoren und deren Verwandtschaft miteinander vermitteln soll.

RÉSUMÉ

Les tumeurs neuro-endocrines qui proviennent embryologiquement de la crête neurale secretent des hormones peptidiques et consistent en tumeurs de cellules carcinoides en tumeurs hypophysaires anterieures en tumeurs thyroidiennes, tumeurs parathyroidiennes chemodectomes, pheochromocytomes et autres Les auteurs ont constaté que leurs fonctions se chevauchent de façon importante et qu'elles sont apparentées plus etroitement qu'on ne le pensait auparavant Les auteurs ont créé un nouveau système de nomenclature pour ce groupe de tumeurs de façon à permettre une compréhension meilleure de ces tumeurs et de leurs relations mutuelles

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presents a confusing perspective regarding them It therefore would seem inappropriate to continue to regard these complex tumors as separate, unrelated entities We believe that some system for grouping them together more closely is needed so that they may be better understood in the future This may be accomplished with a new nomenclature system as follows Key Neuroendocrine tumor (s), location (s) ____, histologic type (s), secreting ____

For instance, some of the tumors from the literature and that we have dealt with in our own experience would be

Case 1 Neuroendocrine tumors, I parathyroid gland and II duodenum, I parathyroid adenoma histologic types, secreting I parathormone, and II carcinoid-islet cell histologic type, II secreting gastrin and insulin

Case 2 Neuroendocrine tumor, pancreas, carcinoid-islet cell histologic type, secreting gastrin and insulin

Case 3 Neuroendocrine tumor, duodenum, carcinoid-islet cell histologic type secreting gastrin

Case 4 Neuroendocrine tumor, pancreas, gastrointestinal tract, lungs, carcinoid-islet cell type secreting gastrin and 5HIAA

Case 8 Neuroendocrine tumor, pancreas, carcinoid islet cell histologic type, non-secreting

Other examples would be Neuroendocrine tumor, bladder, chemodectoma histologic type, secreting catecholamines (22, 24) Another, neuroendocrine tumors, I thyroid and II adrenal medulla, I medullary thyroid carcinoma and II pheochromocytoma histologic type, I secreting ACTH (3)

This nomenclature system would seem to both simplify our thinking about these complex tumors and to stimulate us to consider that a neuroendocrine tumor may either be multifunctional or be associated with other neuroendocrine tumors Therefore, patients with a neuroendocrine tumor should be considered for a complete neuroendocrine analysis

Acknowledgements

Our thanks to Dr G Blackard endocrinologist and Dr Fred Hunter both of the Department of Medicine LSU Medical School, New Orleans, Louisiana for their comments and criticisms towards the production of this paper

SUMMARY

The neuroendocrine tumors which originate embryologically from the neural crest secrete peptide hormones and consist of carcinoid islet cell tumors anterior pituitary tumors thyroid tumors, parathyroid tumors chemodectomas pheochromocytomas and others have been found to possess significant functional overlaps and to be related more closely than was previously thought We have devised a new nomenclature system for this group of

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The effect of ionizing radiation is complex, a fact that may influence the distribution of chromium between different tissues The purpose of the present investigation was to determine what happens to intravenously injected $^{51}CrCl_3$ in irradiated and nonirradiated mice with respect to the distribution of chromium in whole blood, in plasma, and in different protein fractions The distribution at different time intervals after the injection was investigated An attempt was also made to elucidate the fate of those chromium atoms not bound to proteins

Material and Methods

Animals A total of 129 female mice (NMRI strain), 70 to 100 days old, weighing 27 to 30 g, were employed The animals had free access to water and a commercial type pellet diet The abdomen of 63 of the mice was irradiated, the other 66 mice served as controls

Radiation was applied to the abdomen with a ^{60}Co kilocurie unit The SSD (source to skin distance) was 35 cm and from exposure measurements the absorbed dose rate was calculated to be 860 ± 10 rad/min ($\bar{X} \pm SD$) The animals were placed in a perspex holder, which prevented any free movements of the abdominal region, the holder being placed on a lead shield 4 5 cm thick with a 3 cm × 3 5 cm hole that permitted radiation to the ventral aspect of the abdomen Sixty-three animals received 1 000 rad each in doses of 500 rad on two consecutive days, they were conscious during the radiation

Isotope injection and sample collection An isotonic saline solution of $^{51}CrCl_3$ (specific activity 100 to 300 μCi/μg chromium, Amersham, England) was used Each animal was injected with 10 μCi diluted with 0 9 % NaCl to 0 4 ml (about 1 μg chromium/g plasma protein)

Three days after the last irradiation the radioactive solution was injected into the tail vein of each animal The success of the injection was checked by comparing the whole body counts of the animals with the count rate of solution measured under similar geometric conditions At 2, 24 and 48 hours after injection of the chromium tracer, blood samples were withdrawn from the tail vein into heparinized capillary tubes (Scluco Scientific, New York) and centri-

fuged for ten minutes. Plasma samples were collected from 30 animals (15 irradiated, 15 nonirradiated) for investigating the distribution of the tracer among the plasma proteins It was necessary to pool the plasma obtained from 3 animals to obtain the minimum volume required for polyacrylamide gel electrophoresis (about 20 μl)

The activity in whole blood and the distribution of activity between plasma and erythrocytes were determined as follows in 15 animals (8 irradiated, 7 nonirradiated) Twenty four hours after the injection of 0 4 ml $CrCl_3$, blood samples were taken from each animal in heparinized capillary tubes and centrifuged for ten minutes The tubes were then broken just at, or below the border between the plasma and the erythrocytes, each fraction was placed in a plastic vial and 1 ml water added The animals were then killed, 0 5 ml of blood being collected from each animal and the activity determined

The blood volume was assessed in 20 mice (10 irradiated, 10 nonirradiated) with an isotonic solution of ^{131}I IHSA (Amersham, England) Each animal was injected with 0 1 μCi diluted with 0 9 % NaCl to 0 1 ml four days after the irradiation Each injection was checked by comparing the difference in count rates in the syringe before and after injection with that of a standard solution measured under similar geometric conditions About 20 minutes after injection the mice were killed and 0 3 to 0 5 ml blood was collected from each animal in heparinized vials and the activity and the hematocrit of the samples were determined

The activity in the dialyzable part of the plasma was measured 2, 24 and 48 hours after injection in 60 animals (30 irradiated, 30 nonirradiated Fifteen minutes before decapitation the animals were injected with 25 IU (0 5 ml) heparin [illegible]

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Measurements of radioactivity For ^{51}Cr a Well type scintillation counter with a pulse height analyzer set for 270 to 380 keV photons was employed All samples were placed in plastic vials together with a constant amount of solution to avoid corrections caused by volume difference Counting times of 40 minutes for the different protein fractions and 1, 10 and 20 minutes for the other samples proved sufficient to ensure statistical significance ($p < 0.05$) The background activity was measured at least three times on each occasion The minimum detectable number of counts accepted for a single sample was determined according to the following formula

$$r_D = 3\sqrt{r_B / t_T}$$

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$$r_p = 3\sqrt{r_B/t_T}$$

Table 1

Activity in 0 5 ml of whole blood 24 hours after intravenous injection of $^{51}CrCl_3$ (0 4 ml) The plasma activity is expressed as a percentage of the summed activity of the plasma and the blood cells in the capillary tube sample

	No	Activity in whole blood in counts/10 min $\bar{X} \pm SD$	Percentage of activity in plasma $\bar{X} \pm SD$
Irradiated mice	8	$139 \times 10^3 \pm 28 \times 10^3$	97 0 ± 0 6
Nonirradiated mice	7	$141 \times 10^3 \pm 22 \times 10^3$	97 0 ± 0 5

where r_p = the net pulse rate
r_B = the background pulse rate
t_T = counting time for the sample

For ^{131}I the blood volumes were determined with the same scintillation counter, pulses between 330 and 430 keV being accepted The blood samples were counted together with one of known activity for two minutes

Polyacrylamide gel electrophoresis was performed in a discontinuous system by the DAVIS (1964) method at a gel pH of 9 5 Gels were stained with 0 9 % w/v amido black for two hours and destained electrophoretically in 3 % acetic acid They were then sliced into 30 to 35 2 mm pieces The pieces were placed in plastic vials and dissolved in 2 ml hydrogen peroxide 30 % at 80° for six hours and analyzed for activity as described above

Dialysis and chromatography An amount of 0 5 to 1 ml blood was dialyzed against 2 to 4 ml NaCl 0 9 % for ten hours at room temperature The activity of the fluid inside and outside the membrane was measured The solution outside the dialysis membrane was chromatographed on a 1 cm × 90 cm Sephadex G-10 column (Pharmacia Fine Chemicals, Sweden) at a flow rate of 6 ml/h and 2 ml fractions were collected and assayed for activity As a control, 0 8 ml $^{51}CrCl_3$ injection solution (20 μCi) was chromatographed in the same way

Results

Table 1 indicates the total activity of ^{51}Cr in 0 5 ml whole blood in irradiated and nonirradiated animals, no significant difference was evident About 97 0 per cent of the activity lay in the plasma in both groups

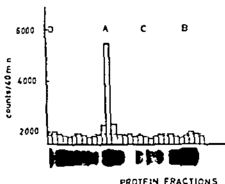


Fig 1 Typical distribution of ^{51}Cr in plasma proteins after 48 hours as separated by polyacrylamide gel electrophoresis The activity in the different samples is plotted against their position in the gel, activity expressed in counts per 40 minutes A transferrin B albumin, C ceruloplasmin, D immunoglobulin - - background level

Table 2

The concentration of ^{51}Cr in the transferrin and albumin bands at different time intervals after the intravenous injection of irradiated and control mice, expressed as percentages of the total activity in the plasma proteins Each value represents the mean for five pools each of three plasma samples

Time interval after inj in hours	Transferrin				Albumin			
	Irradiated		Controls		Irradiated		Controls	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
2	70 6	±6 0	69 0	±9 2	7 8	±10 1	11 3	±3 8
24	91 4	±4 9	92 8	±7 4	1 3	± 1 9	2 1	±2 7
48	93 5	±4 5	95 6	±6 0	1 1	± 1 9	1 0	±2 3

Table 3

Activity in the dialysable part of plasma Samples were taken at 2, 24 and 48 hours after the intravenous injection of 10 μCi $^{51}CrCl_3$ (0 4 ml) The values are expressed as percentages of the total activity in plasma

	Two hours			Twenty four hours			Forty eight hours		
	No	$\bar{X}$	SD	No	$\bar{X}$	SD	No	$\bar{X}$	SD
Irradiated mice	10	11 6	±2 3	10	2 0	±0 5	10	1 0	±0 4
Nonirradiated mice	10	12 3	±4 0	10	1 8	±0 6	10	0 7	±0 4

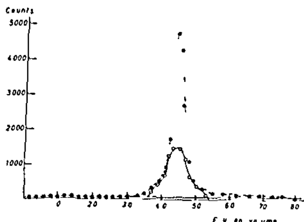


Fig 2 Chromatography on Sephadex G 10 of the injection solution of 20 μCi $^{51}CrCl_3$ and from the dialysate of plasma from a mouse injected 24 hours earlier Only values greater than MDA were accepted The background activity subtracted --- injection solution counts/min plasma sample counts/10 min

The blood volume was 2 3 ± 0 3 ml and the hematocrit 45 ± 1 3 in the irradiated mice The volume in the nonirradiated animals was 2 4 ± 0 3 ml and the hematocrit 45 ± 0 3 The radiation thus failed to produce any significant difference The values are expressed as $\bar{x}$ ± SD

The distribution of ^{51}Cr atoms among the different protein fractions separated by electrophoresis 48 hours after injection is presented in Fig 1 Most chromium atoms, when given in trace amounts, are mainly concentrated in the transferrin band (95 per cent of the total activity of the plasma proteins) Small amounts of activity may also occur in the albumin fraction (2 to 3 per cent) and at the beginning of the gel (1 to 2 per cent) The concentration of ^{51}Cr in the transferrin and albumin bands expressed as percentages of total activity in the plasma protein sample at different time intervals after injection appear in Table 2 No differences between irradiated and nonirradiated animals were evident Two hours after injection about 70 per cent of the total activity was concentrated in the transferrin band, at 24 hours the value had risen to about 92 per cent and this high value persisted at 48 hours

The amount of the ^{51}Cr atoms not bound to plasma proteins appears in Table 3 About 12 per cent of the total activity passed through the dialysis membrane two hours after injection This value decreased to 1 9 per cent at 24 hours and 0 9 per cent at 48 hours There were no differences between the irradiated and nonirradiated animals at the different time intervals

The chromatographic behaviour of the injection solution and the dialysable part of blood plasma are indicated in Fig 2 The peaks of activity were evident in the same eluted volumes The absence of another peak in the chromatogram

of the plasma dialysate probably indicates that the activity atoms that fail to bind to protein also do not become fixed to low molecular substances in blood

Discussion

It is important to know whether ionizing radiation influences the distribution of chromium in the organism This is especially fundamental when $^{51}CrCl_3$ is to be used as a tracer in investigations of irradiated animals The present work clearly reveals that no significant differences in the distribution of chromium atoms in whole blood, plasma and plasma proteins could be demonstrated in irradiated and nonirradiated mice Neither could any effect of irradiation be demonstrated in the distribution observed at 2, 24 and 48 hours after injection

VITTORIO et coll (1963) in an investigation with intraperitoneally injected sodium radiochromate reported that whole-body irradiated mice had a slightly higher ^{51}Cr content in the blood 24 hours after injection compared with nonirradiated mice With $^{51}CrCl_3$ in mice irradiated with 1 000 rad about four per cent more of the activity given was lost by the faeces during the first four days, compared with the nonirradiated mice However, the losses in the urine were much larger (about 15 per cent) in both groups and varied considerably from animal to animal (FRANKENDAL) This might explain why a lower value of activity in whole blood did not occur in the present investigation

A radiation dose of 1 000 rad to the abdomen administered as 500 rad on two consecutive days failed to provoke significant changes in blood volume or hematocrit This is in accordance with the findings of WETTERFORS et coll (1965) in rabbits after whole body irradiation, a reduction in the red cell volume, and a maintenance or an increase of the plasma volume occurred Only a part of the hematopoietic tissues was irradiated in the present series and no changes in the hematocrit were evident Furthermore, this dose does not kill the animals during an observation time of 80 days (FRANKENDAL)

During the first two hours the chromium concentrates in the transferrin fraction and this continues during the next two days At 48 hours about 95 per cent of the activity of the plasma proteins could be demonstrated in the transferrin band However, the binding of chromium to protein is dependent on the amount intravenously injected (COHEN et coll 1965) Trace amounts (1 to 2 μg chromium/g serum protein) are necessary, at higher concentrations (40 μg chromium/g serum protein) the isotope will lie in most of the protein fractions with relatively higher amount of the activity in the albumin fraction

The relative amount of ^{51}Cr in the dialyzed part of the plasma, which is presumed to be free chromium as judged from its chromatographic behaviour on Sephadex G 10, was about 12 per cent of the total activity in blood, two

hours after injection This value had decreased at 48 hours to less than one per cent, the decrease might be partly explained by the excretion of the ions in the urine It has earlier been demonstrated that the amount of activity disappearing in the urine during the first days after injection of $^{51}CrCl_3$ varies between 20 to 30 per cent (ROOTWELT 1966)

The distribution of chromium between the plasma (97 per cent) and the erythrocytes is in good agreement with values obtained from experiments with other species, such as the rat (HOPKINS & SCHWARZ 1964), and the dog (GRAY & STERLING 1950) Chromium has been shown to concentrate in the transferrin fraction in rat (HOPKINS & SCHWARZ 1964) and rabbit and man (COHEN et coll 1965) The same observation has now been made in mice VAN TONGEREN & MAJOOR (1966) have reported that after the intravenous injection of ^{51}Cr-albumin a redistribution of chromium from the albumin to other plasma proteins, especially transferrin occurs The findings of the present investigation are in accordance with this observation

It seems from the present results that injection of trace amounts of $^{51}CrCl_3$ into mice is a useful means of labelling transferrin in vivo As radiation does not influence this labelling, the method may be used to examine the leakage of plasma proteins into the gastrointestinal tract

SUMMARY

The distribution of intravenously injected $^{51}CrCl_3$ was investigated in whole blood plasma proteins and the low molecular part of the blood in irradiated and nonirradiated mice The chromium atoms are selectively bound to the plasma protein transferrin A relative increase in the chromium label occurs during the first 24 hours and 48 hours after the injection the distribution remains unchanged

ZUSAMMENFASSUNG

Die Verteilung intravenös injizierten $^{51}CrCl_3$ wurde im Gesamtblut den Plasmaproteinen und dem niedermolekularen Teil des Blutes von bestrahlten und nichtbestrahlten Mäusen untersucht Die Chromatome waren selektiv an das Plasmaprotein Transferrin gebunden Ein relativer Anstieg der Chrombindung erfolgte während der ersten 24 Stunden 48 Stunden nach der Injektion war die Verteilung unverändert

RÉSUMÉ

Les auteurs ont étudié la distribution du $^{51}CrCl_3$ injecté par voie intraveineuse dans le sang total dans les protéines plasmatiques et dans la partie du sang de faible poids moléculaire chez des souris irradiées et non irradiées Les atomes de chrome sont sélective ment liés à la transferrine des protéines plasmatiques Il se produit une augmentation

relative du marquage au chrome au cours des premieres 24 heures et, 48 heures apres l injection la distribution reste inchangee

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FRACTIONATION SCHEME WITH LOW INDIVIDUAL TUMOUR DOSE AND HIGH TOTAL DOSE

P A JAKOBSSON and B LITTBRAND

The degree of oxygenation varies considerably in neoplastic tissue by reason of the deficient vascular supply Most of the values reported for the proportion of anoxic cells range from 14 to 20 per cent (CLIFTON et coll 1966, HEWITT et coll 1967 REINHOLD 1966 VAN PUTTEN & KALLMAN 1968) The proportion of poorly oxygenated cells is much smaller in normal tissues than in tumours (VAN DEN BRENK 1968) As has been observed experimentally (CLIFTON et coll 1966 HEWITT et coll 1967, REINHOLD 1966, VAN PUTTEN & KALLMAN 1968, SUIT & MAEDA 1966) as well as in carcinoma in man (CATER & SILVER 1960, KOLSTAD 1964 THOMLINSON & GRAY 1955) The lack of homogeneity of the tumour cell population as regards oxygenation and hence radiation sensitivity, constitutes a major problem in radiation therapy

A number of methods have been tried in an attempt to increase the efficiency of radiation therapy in respect of the poorly oxygenated tumour cells, for instance the breathing of 100 per cent oxygen before and during irradiation (HULTBORN

Submitted for publication 23 November 1972

& FORSSBERG 1954), the application of hyperbaric oxygen *during irradiation* (CHURCHILL-DAVIDSON et coll. 1955) and the local injection of hydrogen peroxide (MALLAMS et coll. 1965). Other methods have included the breathing of a mixture of 95 per cent oxygen and 5 per cent carbon dioxide under normal pressure so as to increase the oxygen tension by hyperventilation and capillary dilatation (DU SAULT 1963) and the transfer of all tissue to anoxia by clamping off the extremity, thereby levelling out the differences in sensitivity (BAKER et coll. 1966). Irradiation with high LET has been used when the radiation sensitizing effect of the oxygen has been less marked (BEWLEY 1970), and the technique devised by PIERQUIN (1964) in which a higher dose is applied to the centre of the tumour has been employed.

Observations in various laboratories with a variety of biologic specimens suggest that the cellular repair of sublethal radiation damage is oxygen dependent (BRYANT 1970, HALL & CAVANAGH 1967, HOWARD 1968, KEMBER 1967, LITTBRAND 1970, LITTBRAND & RÉVÉSZ 1969, PHILLIPS & HANKS 1968, SUIT & MAEDA 1970). These results open up other approaches to the solution of the problem of killing poorly oxygenated tumour cells (LITTBRAND & RÉVÉSZ 1970, LITTBRAND 1971); they imply that not only is the well oxygenated normal tissue more radiation sensitive than the poorly oxygenated tumour cells but that *the presence of oxygen endows a protective effect by repair of sublethal radiation damage*. The different capacity of recovery after irradiation influences the shape of the survival curves in the presence and absence of oxygen. The curves approach nearer each other at decreasing dosage and cross at a dose level of about 100 rad (ROBINSON & RÉVÉSZ 1962, LITTBRAND 1970). The capacity for repair of sublethal radiation damage is also impaired for hypoxic cells (less than 1 000 ppm O_2) (LITTBRAND & RÉVÉSZ 1969, RÉVÉSZ & LITTBRAND 1969), i.e. the sensitizing effect of oxygen is dose-dependent at a concentration of 1 000 ppm O_2 or less. The relative difference in survival between well oxygenated normal tissue and poorly oxygenated malignant cells is therefore decreased with a fractionation scheme of low individual doses (BACLESSE 1958). Such a change in the scheme enables the total dose to be raised without necessarily any increase in damage to the normal tissue. On the other hand, the rise in the total dose means that a greater lethal effect will be obtained on the tumour cell populations whose capacity for repair of sublethal radiation damage is impaired or abolished.

A modification of the usual fractionation scheme in accordance with these principles has been tested in a patient with multiple basal cell carcinoma. This was to determine whether with an increase in the total dose combined with a decrease in the individual dose the damage to normal tissue might be kept within the bounds of that produced by the usual fractionation scheme. The

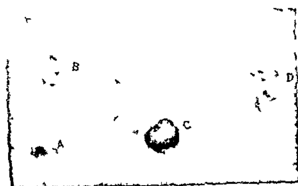


Fig 1 Male aged 81 with four basal cell carcinoma lesions situated dorsally (A B C and D)

choice of total dose and time interval between the individual treatments is dealt with under Discussion.

Material and Method A man aged 81 had for 8 months had four unhealed dorsal lesions (A B C D) A 7, B 11, C 11 and D 14 mm in diameter, respectively All were plane except C which was raised 2 mm (Fig 1) Biopsy of specimens from each of the lesions revealed basal cell carcinoma Roentgen therapy was applied (50 kV, HVL 1 5 mm Al, dose rate 800 rad/min) Three fractionation schemes were used

Fractionated dose (rad)	Total (rad)	Time (days)	Tumours	No of treatments (daily)
(1 10 500	5 000	13	D	1
2 32 × 200	6 400	44	A	1
(3 28 × 3 × 100	8 400	41	B C	3 at 4 h intervals

The patient was treated five days a week in all three schemes

Results

At the end of the radiation therapy tumour D treated according to fractionation scheme 1, displayed mild erythema of the skin which increased gradually and 21 days later was marked and covered the whole area exudative dermatitis appeared at thirty-one days after the therapy (Fig 2)



Fig 2 Tumour A at the end of the irradiation with 6 100 rad 32 fractions over 44 days (fractionation scheme 2) Tumour B and C three days after completion of therapy of 8 400 rad 84 fractions over 41 days (fractionation scheme 3) Tumour D 31 days after termination of treatment with 5 000 rad 10 fractions over 13 days (fractionation scheme 1)

Tumour A, treated according to fractionation scheme 2, displayed mild erythema at 2 800 rad (14 fractions, 19 days) which increased gradually at 5 000 rad (25 fractions 37 days) it was marked with incipient exudative dermatitis At the end of the therapy (6 100 rad in 32 fractions of 44 days) frank exudative dermatitis had developed (Fig 2)

Tumours B and C treated according to fractionation scheme 3, displayed at 3 900 rad (39 fractions, 19 days) mild erythema, which gradually increased until at 7 800 rad (78 fractions, 37 days) it was considerable, the incipient exudative dermatitis at the end of the treatment (8 400 rad, 84 fractions over 41 days) became frank (Fig 2)

In all 4 tumours the dermatitis and all evidence of malignant remnants disappeared after the radiation therapy in tumour A at 27 days, in B and C at 30 days and in D at 52 days The areas of treatment were healed and only mild erythema could be detected in the centre of tumour A at 14 days B 47 days C 51 days and in tumour D at 75 days after the treatment was completed (88 88, 88 and 92 days respectively, after it was begun) Fig 3 depicts tumour A at 73 days, tumours B and C at 76 days and tumour D at 105 days after completion of radiation therapy, no difference was apparent in the healing of the irradiation damage to the normal skin despite the different fractionation schemes The same observation was made 18 months after termination of the treatment

Discussion

The three forms of treatment yielded equivalent results as regards healing of the radiation damage and the tumour reaction to the radiation This was expected so far as a comparison between 10 × 500 and 200 × 32 rad was concerned they would have the same effect according to the STRANDQVIST

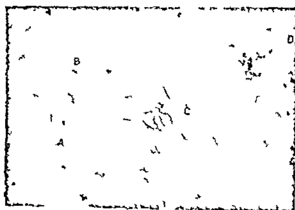


Fig 3 Tumour A 73 days B and C 76 days and D 105 days after completion of irradiation

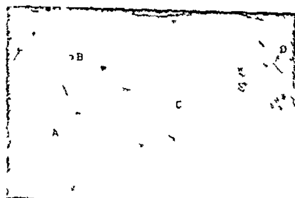


Fig 4 Tumour A B C and D 12 months after termination of irradiation

scheme For a dose fraction of 100 rad the comparison was complicated by the need to consider the magnitude of the tumour dose and the interval between the treatments The total dose may be estimated by any of a number of methods In many exp

he survival

$$S = 1-(1-e^{-D/D_0})^n \qquad (1)$$

where S is the surviving fraction at a chosen dose D, D_0 the dose that lowers the survival by 37 per cent when the survival curve is exponential and n the extrapolated intersection of the exponential curve at zero rad (the extrapolation number) For fractionated treatment the surviving fraction would be

$$S = [1-(1-e^{-D/D_0})^n]^N \qquad (2)$$

where N is the number of treatments If $n = 2$ and $D_0 = 140$ rad, a certain value of S is obtained when a cell population is treated with 200 rad 32 times (that is, $D = 200$ rad an $N = 32$) This value of S may be regarded as a relative measure of the effect of the oxygenated *normal tissue An estimate of the total* dose with the same effect on normal tissue when the tumour dose is 100 rad may be made by putting the same value of S in the formula, *giving D_0 and n the* same value as before but with $D = 100$ rad and solving the expression for N The number of fractions should exceed 80 — that is to say the total dose should be greater than 8 000 rad The same figures were obtained in a comparison performed by the STRANDQVIST (1944) or FOWLER (1965) schemes and in a calculation by means of the ELLIS (1969) formula No account is taken with formula II of the time interval between treatments On the other hand, the other three bases for the calculation presuppose that the therapy is given once a day, which entails a long session for the patient Because of the uncertainty whether *the tumour growth during the treatment could be controlled by a single* tumour dose of 100 rad a day (MALAISE & TUBIANA 1966, HERMENS & BARENDSEN 1969), *the possibility of giving the treatment on several occasions on the same day* was considered (SIMPSON 1970) Repair of the radiation lesions in the surrounding normal tissue is required between each treatment Two types of repair are conceivable, namely repair of the intracellular sublethal damage, and restoration of tissue by repopulation Investigations with the skin of pigs conducted by FOWLER et coll (1963) indicated that the healing effect is predominantly an intracellular repair, repopulation being of only minor importance The time required for the intracellular repair is only a few hours (ELKIND & WHITMORE 1967) It appears that a radiation dose synchronizes the cell population with the result that the cells vary in sensitivity at the next treatment depending on the phase in the cell cycle (ELKIND & SUTTON 1960, HORNSEY & VATISTAS 1963, TERASIMA & TOLMACH 1963) *Such work has demonstrated* fairly consistently that the survival reaches a maximum about 4 hours after the first treatment, falls to a minimum at about 6 hours, and again attains a maximum on a plateau at about 12 hours or more Whether this applies also to the treatment given as extremely small doses and whether to over a complete series of treatments has not been examined experimentally These results, however, indicate that for the minimum effect on the normal tissue surrounding the tumour the interval should be 4 hours or more over 12 hours

All these theoretic calculations suggest that the most promising approach is the fractionation scheme consisting *of 84 treatments, each of 100 rad given* 3 times a day at intervals of 4 hours, this should produce the same damage to the normal tissue as *treatment* with 200 rad each day and a total dose of 6 400 rad The result of the treatment of the patient with basal cell carcinoma suggests that the

fractionation scheme with 84 × 100 rad does not cause more severe injury to normal tissue than 32 × 200 or 10 × 500 rad, this applies both to immediate healing of the radiation injury and changes in the skin 18 month after the treatment is completed

Support for these conclusions lies in the fact that the treatments were performed in the same patient, and the tumours had the same location, extent and pathologic features The investigation does not, however, indicate whether any individual variations existed between patients in a comparison between the various schemes Nor do the results afford any insight into the occurrence of late damage to, for instance connective tissue, muscle, nervous tissue or vessels, or the occurrence of late damage when large tissue volumes are treated Preliminary indications in the treatment of tumours of the oral cavity, however, suggest that a total dose of 8 400 rad is well within tolerable limits if the described fractionation scheme be applied (BACKSTROM et coll, to be published, JAKOBSSON et coll 1971)

Acknowledgements

The authors express their sincere thanks to Prof Laszló Revesz and Dr Oliver Scott for their useful suggestions

SUMMARY

A modified fractionation scheme with low individual doses has been tested on a man with multiple basal cell dorsal carcinoma A total dose of 8 400 rad was delivered with three treatments daily *each of 100 rad* The local healing of the irradiated tissue was compared to that obtained when two other fractionation schemes were used as controls and proved not to differ

ZUSAMMENFASSUNG

Ein modifiziertes Fraktionierungsschema mit niedrigen E

RÉSUMÉ

Les auteurs ont essaye une schema modifie de fractionnement avec de petites doses partielles pour le traitement chez l homme de carcinomes baso-cellulaires dorsaux multiples Une dose totale de 8 400 rad a ete administree avec trois traitements par jour, de chacun 100 rad

La guérison locale du tissu irradié a été comparée à celle qui est obtenue avec l'utilisation de deux autres schémas de fractionnement utilisés comme témoins, le résultat n'est pas différent

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ELECTRON IRRADIATION OF MONKEY SKIN

S W LIPPINCOTT, J L MONTOUR and J D WILSON

An astronaut working in a space suit outside of a shielded vehicle, positioned either in the outer belt or in the synchronous orbit, might readily be exposed to low energy electrons that normally occur in those regions To determine what the hazards might be, a series of experiments simulating such conditions have been undertaken in the monkey irradiated with 0 5 to 1 75 MeV electrons Both mono- and polyenergetic irradiations have been employed In this report a correlation has been made of the early responses in the skin of the monkey with reference to depth dose pattern, size of area involved, and post irradiation periods Extrapolation from such animal data to man is not expected to provide an absolute answer to the problem, however, it should produce a reasonable basis for helping to establish a maximum permissible dose for future astroscientists in the aforementioned situation

Methods and Materials Nineteen rhesus monkeys were irradiated with low energy electrons ranging from 0 5 to 1 75 MeV Both mono- and polyenergetic irradiations were performed The electron spectrum of the equatorial synchronous orbit was satisfactorily simulated with the use of three monoenergetic

Submitted for publication 15 May 1972

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Table

Clinical observations

Animal No	Dose (rad)	Time lesions first observed		
		Wet desquamation	Erythema	Ulceration
Monoenergetic trunk irradiation 0 95 MeV				
1	450	—	—	—
49	540	—	—	—
11	550	—	—	—
47	800	—	—	—
11	830	—	—	—
2	900	5	12	—
34	960	—	12	—
47	1 040	5	—	—
48	1 130	5	—	—
49	1 260	5	13	—
48	1 450	13	13	14
3	1 510	5	—	19
35	1 520	12	17	18
4	1 800	11	—	13
Monoenergetic trunk irradiation 0 5 MeV				
6	1 040	12	—	—
5	1 800	12	—	19
Monoenergetic spot (3 cm × 8 cm) irradiation 0 95 MeV				
33	950	22	—	—
45	1 150	—	22	—
33	1 470	—	22	—
45	1 890	—	15	—
Spectral trunk irradiation 1 75 1 30, and 0 95 MeV				
9	870 (140 170 560)	—	—	—
8	1 554 (239 405, 910)	12	12	21
10	1 790 (330, 410 1 050)	12	—	23
32	2 900 (790, 710, 1 400)	—	17	20
7	2 993 (810 625, 1 558)	12	—	12

MeV and ulceration occurred at 1 800 rad by the nineteenth day To compare the effects upon a much smaller area (3 cm × 8 cm) 0 95 MeV energy was again used and wet desquamation was noted by the twenty second day at 950 rad Erythema was seen at 1 150 rad on the twenty-second day and on the fifteenth day at 1 890 rad Ulceration did not occur at these doses, whereas, it had in the larger trunk irradiations at 1 450 to 1 800 rad

The spectral trunk irradiations were made at 1 75, 1 30, and 0 95 MeV in an

electron beams incident on the target animals The area sizes irradiated were 3 cm × 8 cm, half trunk and whole trunk, all with the monkey covered by standard astronaut space suit material (equivalent to 2 mm of tissue), except at the lowest energy The penetration at these energies is such that only skin and subcutaneous tissue are involved, even at the maximum energy employed The absorbed doses given are those on the skin surface of the monkey Several dosimetry systems were used, as described by us before (LIPPINCOTT et coll 1970) to determine the depth dose characteristics A tissue equivalent extrapolation ion chamber was used to measure tissue dose at various depths in tissue equivalent plastic under the same irradiation conditions as for the animal exposures Thermoluminescent dosimeters and an NBS-calibrated 1 cm^3 tissue equivalent ion chamber were used in beam uniformity measurements and as reference monitors for the various exposures A Faraday cup receiving the straight ahead electron beam was used to obtain consistency of performance of the electron accelerator

Results

In the clinical examination of monkey skin it must be borne in mind that alterations do not necessarily occur precipitously, and that there is not regularly an exact sequence of events with any given dose capable of provoking a reaction Thus, there may be weak or strong reactions which determine whether it is difficult or relatively easy to distinguish the various lesions Individual animals may differ in reactions to the same dose In general, oozing or mild wet desquamation has to be searched for quite carefully and palpating the skin frequently gives the earliest clue Erythema varies from a light pink to a reddish shade and may or may not be preceded by oozing or wet desquamation Ulceration is the most marked entity and easily recognized From a practical point of view, clinical examinations three times a week for the first three weeks and once a week thereafter, up to four months, were found to be sufficient for a careful follow-up of each monkey Biopsies were taken from each animal before and at the time of appearance of the various lesions

Monoenergetic trunk irradiations were carried out at 0 95 MeV giving a penetration of 1 4 mm, thus extending to the subcutaneous tissue Fourteen different doses were administered from 450 to 1 800 rad as indicated in the Table Mild wet desquamation occurred at a dose as low as 900 rad and as early as five days post-irradiation Erythema was observed at 900 rad by the twelfth day Ulceration did not develop at a dose lower than 1 450 rad or earlier than the fourteenth day Healing in this instance was complete by the forty-second day In addition to the above ten monkeys, two were similarly irradiated but at 0 5

from the three species The mouse data are probably the least helpful in attempting to determine a maximum permissible dose based on observations up to four months On the matter of life time follow-up for possible carcinogenesis in the mice our investigation at this time is not as yet completed With monoenergetic trunk irradiation erythema was observed in the monkey by the twelfth day at 900 rad and ulceration at 1 450 rad on the fourteenth day while for the pig the lowest dose for production of erythema was 1 340 rad at forty-three days and ulceration at 2 650 rad at twenty-one days With spectral trunk irradiation in the monkey erythema occurred at 1 554 rad at twelve days and ulceration at twenty-one days while for the pig erythema occurred at 1 680 rad at 50 days and ulceration did not develop in doses up to 2 450 rad

Conclusion

Nineteen rhesus monkeys were irradiated with low energy monoenergetic and spectral electrons simulating conditions as they exist in the outer belt and the equatorial synchronous orbit A correlation of the early pathologic responses in the skin was made with reference to depth-dose pattern, size of area involved, and post irradiation observation periods With monoenergetic trunk irradiation wet desquamation occurred as early as five days at 900 rad, erythema at twelve days at 900 rad, and ulceration at fourteen days at 1 450 rad When the area irradiated in the same way was only 3 cm $\times$ 8 cm wet desquamation was found at 22 days at 950 rad and erythema at 1 150 rad at twenty two days Ulceration did not occur at doses up to 1 890 rad With polyenergetic trunk irradiation erythema was observed with a total dose of 1 554 rad (239, 405, 910) at twelve days and ulceration at twenty-one days

SUMMARY

Nineteen rhesus monkeys were irradiated with low energy monoenergetic and spectral electrons simulating conditions as they exist in the outer belt and the equatorial synchronous orbit A correlation of the early pathologic responses in the skin was made with reference to depth-dose pattern size of area involved, and post irradiation observation periods The lowest dose producing erythema was 900 rad by five days and for ulceration 1 450 rad at fourteen days

ZUSAMMENFASSUNG

Neunzehn Rhesusaffen wurden mit monoenergetischen Elektronen niedriger Energie und Spektralelektronen bestrahlt, wobei die Bedingungen simuliert wurden wie sie in der ausseren Zone und der aquatorialen synchronen Umlaufsbahn vorliegen Eine Korrelation

elapsed time of forty-five to seventy minutes The Table gives the total doses for each animal as well as the three component doses at the various energies No reaction occurred at 870 rad At 1554 rad erythema was found on the twelfth day and ulceration on the twenty-first day At 1 790 rad mild wet desquamation occurred at the twelfth day and ulceration by the twenty-third day The response of the two animals exposed to total doses of about 3 000 rad showed wet desquamation and ulceration as early as twelve days in one monkey with erythema at seventeen days and ulceration at twenty days in the other

Discussion

In the monkeys irradiated with mono- and polyenergetic electrons it has been *found that dose, size of area exposed, and times at which post-irradiation* observations were made were all factors in reference to the type and extent of lesions that occurred From a practical point of view in trying to extrapolate such data to man it is of interest to know what results would develop with similar radiation experiments in mice and pigs The principal reason for this is that, although of similar structure, the thickness of epidermis and dermis in each of these species is different with that of man being somewhere between monkey and pig and more like the latter

In unpublished data from our laboratory eight groups of ten mice each were irradiated over a 2 cm × 2 cm area with 0 5 MeV electrons, giving a depth of penetration of 1 2 mm The doses ranged from 260 to 12 500 rad Wet desquamation did not occur and in our experience erythema cannot be recognized in mouse skin Greying of the hair is, however, one of the frequent and early findings It was first seen at 25 days at a dose of 1 070 rad Later, by one hundred and thirty days, it was seen at a dose as low as 260 rad Minimal necrosis of skin occurred at 890 rad by the fourth month post-irradiation and frank ulceration at thirty-nine days at 2 240 rad Ulceration at 6 100 rad occurred by the twenty-fifth day and at 12 500 rad by the fourteenth day

In an extended investigation, still underway in our laboratory, *fifty eight* miniature pigs have been irradiated with low energy electrons (LIPPINCOTT et coll [illegible] (2 1 MeV)
at 1 [illegible] ectral trunk
exposures erythema *did* not occur *until 1 680 rad at 50 days* With the former irradiation, ulceration was found at 2 650 rad at twenty-one days and required about four months *for complete healing* No ulceration occurred in the spectral trunk irradiated group up to the highest administered dose of 2 450 rad

In evaluating *the data concerning* early responses to low energy irradiation for the mouse, monkey, and pig in reference to man it is helpful to have information

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DISTRIBUTION OF ^{226}Ra IN THE BONES OF MICE

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Longterm biologic investigations with incorporated bone seeking radionuclides are mainly directed at the production of osteosarcomas (FINKEL et coll 1969, HUG et coll 1969, NILSSON 1970, KLENER et coll 1973) The incidence of bone tumours further depends on the applied dose and the method of radionuclide administration In mice these growths are predominantly localized in the axial skeleton and long bones (EVANS et coll 1944, HOPKINS et coll 1966, SVOBODA et coll 1971) MARSHALL & FINKEL (1959) in endeavouring to explain the differences in localization of osteogenic sarcomas after incorporation of ^{45}Ca, ^{90}Sr and ^{226}Ra carried out a comprehensive autoradiographic dosimetry of the bones of mice The conclusions of this investigation have become topical recently as possible sites of osteosarcomas are becoming more specific This was also aided by ICRP publication No 11 (1968) and recent experiments by BARNES et coll (1970) and LOUTIT & VAUGHAN (1971) designed to elucidate the nature of osteoprogenitive cells. Specification of the skeletal regions where the cells with the highest proliferative potential are present and where the hazards of carcinogenesis could occur requires a more detailed analysis of the nonuniform distribution of activity and dose The present experiments were designed to determine the initial deposition of ^{226}Ra in different regions of the

Reported in abbreviated form at the IRPA Second European Congress on Radiation Protection Budapest 3 to 5 May 1972 Submitted for publication 31 August 1972

der frühen klinisch-pathologischen Reaktionen der Haut wurde im Hinblick auf die Tiefen-Dosis-Verhältnisse, die Grosse des betroffenen Gebietes und die Beobachtungszeiten nach der Bestrahlung vorgenommen. Die niedrigste Dosis, bei der ein Erythem auftrat, betrug 900 rad nach 5 Tagen, und bei der eine Ulceration auftrat, 1 459 rad nach 14 Tagen.

RÉSUMÉ

Dix-neuf singes rhésus ont été irradiés par des électrons mono énergétiques de faible énergie et par des électrons spectraux simulant les conditions qui existent dans la ceinture extérieure et sur l'orbite équatoriale synchrone. Les auteurs ont établi une corrélation entre la résponse anatomo-clinique précoce de la peau et le type de dose en profondeur, les dimensions de la surface irradiée et la période d'observation après irradiation. La dose la plus faible qui produit un érythème est de 900 rad en 5 jours et celle qui produit une ulcération est de 1 450 rad en 14 jours.

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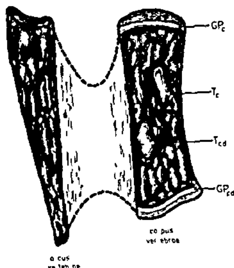


Fig 2 Idealized presentation of lumbar mouse vertebra on a roentgenographic basis GP_{cr} – growth plate in the cranial part of the vertebral body T_{cr} = trabecular bone in the cranial part of the vertebral body T_{cd} = trabecular bone in the caudal part of the vertebral body GP_{cd} = growth plate in the caudal part of the vertebral body

The microdistribution of activity in the growth plates, trabecular bone and compacta of the same mouse was examined in the L2 and L4 sagittally dissected lumbar vertebrae as well as in the longitudinally dissected right femur by means of SST quantitative autoradiography The organic triacetate cellulose foils were exposed 48 hours in direct contact on plane bone slices, tracks being developed by the method of PRICE et coll (1962), the alpha tracks in the selected areas (Figs 1, 2) were evaluated by direct track count The values from 10 fields each 3 600 μm^2 in size and divided into 36 squares were taken for statistical analysis The diffusely localized alpha tracks were counted in the entire plate in those plates that were 90 to 110 μm thick in the vertebra and 114 to 125 μm thick in the femur The alpha tracks in the trabecular bone of the vertebrae and femora were determined in each single trabecula on the side facing the endosteum The alpha tracks in the femoral diaphysis from the compacta were counted on the side facing the endosteum and periosteum and their mean was also calculated (region D, Fig 1), the localization of the tracks was in some experiments verified by the superposition with the histologic sections

Evaluation of the dose rate and accumulated dose

Evaluation of the radiochemical data The average dose rate in bones treated radiochemically was ascertained under the assumption that in vivo the alpha radiation from the radium series is homogenically absorbed by 100 per cent

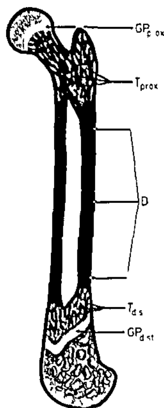


Fig 1 Idealized presentation of mouse femur on a roentgenographic basis GP_{prox} = growth plate in proximal epiphysis T_{prox} = trabecular bone of proximal epiphysis D = diaphysis of femur, T_{dist} = trabecular bone of distal epiphysis, GP_{dist} = growth plate in distal epiphysis

femur and lumbar vertebrae in mice together with microscopy dose distribution examinations at various times after single injections, both radiochemical and autoradiographic techniques were used

Material and Methods One hundred SPF female mice of random bred strain H, 6 to 8 weeks old weighing approximately 25 g were intravenously and intraperitoneally injected with 0 75 μCi ^{226}Ra as chloride in 0 1 ml isotonic solution at pH 3—4 with a calcium chloride carrier After gamma spectrometric measurement in vivo (LENGER 1971) the animals were killed by exsanguination from the subclavian artery in groups of five 30 min, 2 h, 6 h, 12 h, 24 h, 48 h, on the 7th day, 14th day, 21st day and 49th day after injection of the radionuclide Both femora and four lumbar vertebrae were removed

The activity of ^{226}Ra was ascertained radiochemically by determining the Ra/Ca ratio in L1 and L3 as well as in the left femur divided into diaphysis and proximal and distal epiphyses The femur was dissected, assisted roentgenographically, so that the diaphysis would be free from admixture of trabecular bone from both epiphyses (Fig 1)

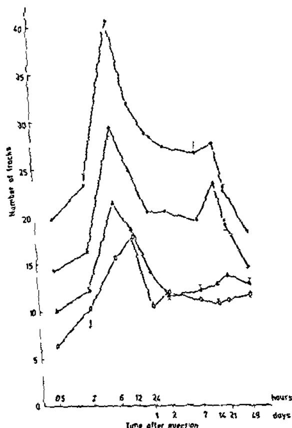


Fig 4 Time course of track densities (number of tracks per 60×60 μm) in mouse femur as certained by SST autoradiography in organic foil (95 per cent confidence limit of the mean is indicated) ○ = growth plate in distal epiphysis ● = growth plate in proximal epiphysis ▲ = trabecular bone of distal epiphysis □ = diaphysis of femur

dose decreases from the initial value of 5 per cent for 0.5 h after application to 2 per cent for the 49th day after application

Evaluation of the autoradiographic data Alpha spectrometry of thick sources disclosed that only 10 *per cent radon is* retained in the bone in vitro (LENGER, personal communication) compared *with the* value of 30 per cent assumed by MARSHALL & FINKEL (1959) Under the assumption that the effectiveness of detection by emulsion autoradiography is 100 per cent in the spatial angle 2 π agreement has been obtained *for specific* ^{226}Ra activity determined radio-

tivity was carried out by

autoradiography calibrated *by determination* of the transformation factors ω of the number of tracks *in the emulsion* per number of holes in the dielectricum

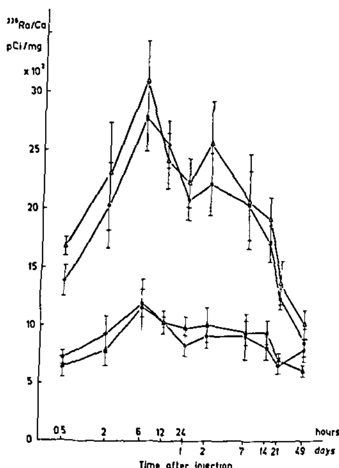


Fig 3 Time course of ^{220}Ra activity distribution in the lumbar vertebra and femur in mouse radiochemically ascertained △ = vertebra lumbalis ● = distal epiphysis of femur, ○ = proximal epiphysis of femur ▲ = diaphysis of femur The 95 per cent confidence limit of the mean is stated at each point

within the bone Recalculation to wet tissue from the Ra/Ca ratio was carried out by means of the experimentally determined factor γ (mgCa/g tissue)

		γ
Femur	Diaphysis	170
	Distal epiphysis	120
	Proximal epiphysis	140
Vertebra		80

The retained radon fraction in vivo was considered by a function of 0 05 $t^{0.15}$ and the initial radon increase approximately by a factor 1-$e^{0\ 18t}$, where t is expressed in days (MAYS et coll 1958)

The dose was calculated from the dose rate by numeric integration The values were brought to normal for application activity 1 μCi/mouse According to the law of propagation of errors it was ascertained that the relative error for the

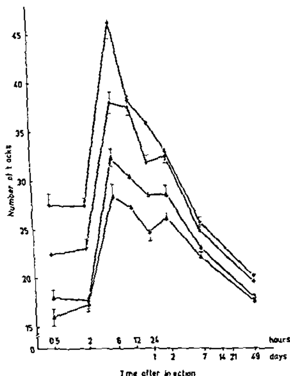


Fig 5 Time course of track densities (number of tracks per 60×60 m) in mouse vertebra lumbalis ascertained by SST autoradiography in organic foil (95 per cent confidence limit of the mean is indicated) O = growth plate in the caudal part of the vertebral body ● growth plate in the cranial part of the vertebral body △ = trabecular bone in the cranial part of the vertebral body ▲ trabecular bone in the caudal part of the vertebral body

is higher after intravenous injection In consideration of the insignificant differences between the two methods of application during the further time the experimental values were averaged for later use Specification by means of quantitative autoradiography disclosed that local [illegible]Ra retention in the femur (Fig 4) also reaches maximum six hours after injection, this is particularly so in the growth plates amounting to somewhat less in the distal trabecular bone whereas in the compact bone of the diaphysis the maximum is recorded twelve hours after injection The time course of the density of tracks in the proximal trabecular bone, which is not demonstrated in the graph, is practically identical with the values in the diaphysis excepting for the maximum 6 hours after the injection The maximum was six hours after injection in the vertebra lumbalis in all the areas investigated (Fig 5)

A somewhat higher 95 per cent confidence limit of the mean occurring in some values of the time course of distribution of track densities in the femur is given by the smaller number of samples in comparison to vertebrae due to the

under otherwise identical conditions. The range of the alpha tracks from ^{226}Ra was assumed to amount to 27.5 μm for reference density of the bone $\varrho = 1.5$ g/cm^3. Recalculation to bone in vivo was carried out by means of the experimentally determined ratio of densities $\sigma = (\varrho \text{ in vitro})/(\varrho \text{ in vivo})$.

		w	ϱ	σ
Growth plates (femur, vertebra)		4.5	1.1	0.79
Femur	Trabecular bone—prox. epiphysis	3.8	1.2	0.81
	Trabecular bone—dist. epiphysis	3.8	1.3	0.81
	Compact bone—diaphysis	4.3	1.5	0.81
Vertebra	Trabecular bone	3.8	1.3	0.81

The dose rate and the accumulated dose were estimated (1) for cells in the growth plates with assumption of 4 π geometry, (2) for the layer of endosteal cells of 10 μm thickness adjacent to the trabeculae in the marrow cavity of 100 μm diameter with assumption of cylindric geometry, and (3) for the layer of endosteal or periosteal cells of 10 μm thickness adjacent to the compact bone of the diaphysis with assumption of plane geometry.

The geometric factors for alpha emitters of the radium series and for the dimensions stated were obtained by interpolation from tables by HOWARTH (1965).

Results

Figure 3 indicates the time course of the specific activities (Ra/Ca ratio) in the diaphysis, distal epiphysis, proximal epiphysis and vertebrae, obtained radiochemically. The individual points are the mean values from 10 mice after the intravenous and intraperitoneal application of ^{226}Ra, it appeared that the difference in the bones between both methods of application does not average more than ten per cent in the entire time course tested by analysis of variance. The diagram illustrates that the maximum levels of incorporated nuclide are attained six hours after application. The activity level in the distal femoral epiphysis and in vertebrae reached within this time period up to three times the values for the diaphysis and the proximal femoral epiphysis. The gradual decline later in all the parts observed, especially in the distal femoral epiphysis and vertebrae, is evident.

The results of the SST autoradiography are presented in figures 4 and 5 as densities of tracks only, proportional to specific activities. The differences between intravenous and intraperitoneal injection of the radionuclide may be proved half an hour to six hours later: during this time period the ^{226}Ra level in bones

Table 1

The doses (rad) accumulated from 1 μCi ^{226}Ra injected. Calculated from data of the radiochemical analysis of bones and from gamma spectrometric whole body counting of mice

Time after injection	Vertebra lumbalis (corpus)	Femur			Skeleton (WBC)
		Epiphysis		Diaphysis	
		Prox	Dist		
0.5 h	0.5	0.4	0.6	0.4	0.4
2 h	3.9	2.8	4.8	3.0	3.8
6 h	16	12	21	12	16
12 h	38	29	52	30	38
1 d	85	64	120	69	83
2 d	170	130	250	135	160
7 d	540	400	730	450	500
14 d	1050	820	1400	910	930
21 d	1500	1200	2000	1300	1300
49 d	2800	2500	3700	3000	2600

Table 2

The dose rates (rad/day) in selected areas of vertebra lumbalis and femur of mice from 1 μCi ^{226}Ra injected (with SST autoradiography)

Time after injection	Vertebra lumbalis				Femur				
	Growth plates		Trabecular bone		Growth plates		Trabecular bone		Compact bone diaphysis
	Cran	Caud	*Cran*	*Caud*	*Prox*	Dist	Prox	Dist	
0.5 h	78	97	*33*	*29*	50	69	12	18	12
2 h	81	95	*37*	*31*	57	81	14	22	16
6 h	133	161	58	51	105	143	36	40	25
12 h	132	135	55	49	88	112	32	33	29
1 d	114	129	51	45	73	103	21	26	17
2 d	121	123	52	50	76	101	20	22	17
7 d	105	109	52	47	62	112	25	27	22
14 d	–	–	–	–	108	127	31	31	24
21 d	–	–	–	–	92	109	28	32	26
49 d	101	104	50	49	69	93	32	37	29

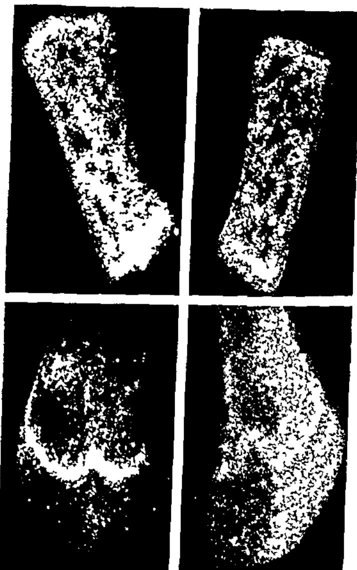


Fig. 6. SST autoradiography of the lumbar vertebra (above) and femoral distal epiphysis (below) of the same mouse 6 hours (left) and 49 days (right) after injection of [illegible]Ra.

technical difficulties connected with obtaining the ideal longitudinal section through out the whole femur.

Comparing the time course of track densities in the trabecular bone of the femur with the same structure in the vertebrae (Fig. 6) the analysis of variance has indicated that the activity beneath the endosteum of vertebral trabecular bone is 1.6 ± 0.3 (95 per cent confidence limit) times higher than in the trabecular bone of the distal epiphysis. It was ascertained furthermore that the variance of the track densities in the trabecular bone of vertebrae is significantly higher than in the trabecular bone of the distal epiphysis of the femur. The

of their application. Experiments in dogs with ^{226}Ra (SEARS et coll. 1963) and with ^{90}Sr (ATHERTON et coll. 1968) as well as with ^{45}Ca in miniature pigs (POND et coll. 1969) disclosed different distribution of nuclides in spongy bone represented by the vertebrae and epiphyses of long bones compared with compact bone represented by the femoral diaphyses. Of all the bones examined the vertebra and the distal femoral epiphysis proved to have the maximum specific activities, especially evident soon after incorporation. This seems to be connected with the different metabolic activity of the particular bone structures in relation to skeletal maturity, it correlates for example in the region of the distal epiphysis of the mouse femur with the closing of the growth plates that occurs as the last.

Spongy bone indicates generally higher metabolic activity than compact bone. In agreement with the authors mentioned and with regard to the ossification process in mouse bones (JAFFE 1931, NEJEDLY 1965) it could be proved by radiochemical methods that the maximum ^{226}Ra deposition is concentrated in the distal epiphysis of the femur and the vertebra at the sixth hour after injection. Simultaneously it was verified that the most significant differences between intravenous and intraperitoneal injection occurred soon after administering the radionuclide, the deposition after intravenous injection was higher.

Some increase of activity in the vertebra and in the distal femoral epiphysis observed on the second day after injection is difficult to be explained by differences in the applied activity (Fig. 3). The values of the whole body counting of mice killed in the corresponding time intervals also fail to disclose such deviation.

Radiochemical methods afford only mean values from the entire bone region examined, a reason why the present authors concentrated rather upon autoradiography. A great experimental investigation was performed in this field by MARSHALL & FINKEL (1959) in which the distribution of ^{226}Ra activity in mouse skeleton was determined by means of histoautoradiography with the classical technique. A consideration of the distribution of activity with special concern to earlier and more frequent intervals after injection in the present work enabled more detailed topographic information on the localization of radionuclide in selected areas of mice bones to be obtained with the SST autoradiographic technique.

In contrast to classical autoradiography based on photographic reduction of the argent bromide grains in the emulsion layer, SST autoradiography is based on detecting heavy particles by means of chemical etching in organic materials, such as of cellulose. This method reveals only heavy particles and the background is very low because of the lower sensitivity to other kinds of energetic

Table 3

The doses (rad) in selected areas of vertebra lumbalis and femur of mice from 1 μCi ^{226}Ra injected (with SST autoradiography)

Time after injection	Vertebra lumbalis				Femur				
	Growth plates		Trabecular bone		Growth plates		Trabecular bone		Compact bone, diaphysis
	Cran	Caud	Cran	Caud	Prox	Dist	Prox	Dist	
0.5 h	0.8	1.0	0.3	0.3	0.5	0.7	0.1	0.2	0.1
2 h	5.8	6.9	2.4	2.2	3.9	5.4	0.9	1.4	1.0
6 h	24	28	9.8	8.9	17	24	5.1	6.5	4.4
12 h	57	65	24	21	41	56	14	16	11
1 d	120	130	50	45	81	110	27	31	23
2 d	240	260	100	93	160	210	47	54	42
7 d	800	840	360	340	550	740	160	180	150
14 d	—		—	—	1200	1600	250	380	310
21 d	—	—	—	—	1900	2400	560	610	480
49 d	5100	5300	2500	2400	4200	5200	1400	1600	1200

ratio of standard deviations of the track densities equals 2 with 95 per cent confidence limits of 1.6 and 2.3

The time course of accumulating doses in the vertebral body and in both femoral epiphyses and the diaphysis obtained by the radiochemically analyzed bones is presented in Table 1, values of the average dose in the mouse skeleton, based on the results of whole body counting, are also included. Table 2 demonstrates the time course of dose rates in the epiphyseal plates of vertebrae and femur, in the endosteal surface of the trabecular bone of vertebrae and of distal femoral epiphysis and finally in the endosteal—periosteal surface of the compact bone of the femoral diaphysis, while Table 3 deals with the doses accumulated in the same areas

Discussion

Considerable attention of the biologic effect of bone-seeking radionuclides has been paid to their nonuniform distribution after incorporation. The numerous kinetic investigations (STOVER 1959, ELLSASSER et coll. 1969, LINITCKI 1971) revealed that the deposition of nuclides depends on many factors, such as the age and species of the experimental animals, the type of bones and the means

of bone tissue The nuclide distribution is also influenced by the mode of administering the radionuclide because of the known difference in the production of neoplasia after the single and multiple application of ^{32}P (BENSTED et coll 1961) and ^{90}Sr (FINKEL 1958)

Knowledge of the distribution of activities and doses in bones affords only a partial aspect of the complicated problems of neoplastic bone localization It would appear important in the further detection of the causes of radiation oncogenesis of osteosarcomas to pay attention to the compartment of distinct populations of bone cell precursors at risk of effective irradiation This together with more real knowledge of microdistribution of activities and doses in the early and late period after application might contribute to the clarification of the relation between dose and effect

Acknowledgement

The authors are greatly indebted to Olga Horáčková for the radiochemical evaluation of the samples Statistical evaluation and analysis was carried out by Eng Zdeněk Roth of the Department of Mathematical and Statistical Methods and Programming who is particularly thanked

SUMMARY

The time course of ^{226}Ra distribution was investigated in separated parts of the femora and vertebrae of young female mice after single injections of 0 03 μCi/g body weight by radiochemistry and SST autoradiography Quantitative autoradiography afforded more precise data than radiochemistry The data were used for dose determinations, especially in the growth plates and in the trabecular and compact bones

ZUSAMMENFASSUNG

Der Zeitverlauf fur die ^{226}Ra Verteilung wurde in gesonderten Teilen der Femura und Wirbeln von jungen weiblichen Mausen nach einer einmaligen Injektion von 0 03 μCi/g Korpergewicht radiochemisch und SST autoradiographisch untersucht Die quantitative Autoradiographie fuhrte zu genaueren Daten als die Radiochemie Die Daten wurden fur Dosisbestimmungen verwendet besonders in den Zuwachsplatten und der trabekularen und kompakten Knochensubstanz

RÉSUMÉ

L evolution dans le temps de la distribution du ^{226}Ra a éte etudiee dans des parties separees de femurs et de vertèbres de jeunes souris femelles après des injections uniques de 0 03 μCi/g de poids corporel au moyen de la radio-chimie et de l auto-radiographie par SST L autoradiographie quantitative a donnee des resultats plus precis que la radio chimie Ces resultats ont éte utilises pour des determinations de doses, en particulier dans les cartilages de conjugaison et dans l os spongieux et compact

activation, such as visible light, roentgen, gamma and beta irradiation. The scanning of alpha tracks is also more simple with the SST technique.

In accordance with the results from the radiochemical analysis, the maximum of activity incorporated was ascertained by SST autoradiography in the bone areas at 6 hours after the ^{226}Ra application; in the femoral diaphysis (Fig. 4) it appeared 12 hours after injection. This may be explained by the better resolution of ascertaining the localization of activities.

The SST technique disclosed further interesting facts not previously described in detail in the literature. The absolute activity in the areas in the vertebra — cranial and caudal growth plates, cranial and caudal trabecular bone (Fig. 5) — is higher than in similar areas in the femur—distal and proximal growth plates, distal and proximal trabecular bone in both epiphyses (Fig. 4). The 1.6 ± 0.3 times higher value with a 95 per cent confidence limit is significant in the cranial part of the trabecular bone of the vertebra in comparison to that of the distal femoral epiphysis. The report by MARSHALL & FINKEL suggests an indication of higher absolute activity in the vertebra compared with the trabecular bone of femur. The lowest activity values were ascertained during the entire time course in the femoral diaphysis in the side facing the endosteum as well as the periosteum. Due to the differences in the metabolism of the bone tissue however the data from the diaphysis may be considered distinct as regards the character of the compact bone of which it is formed.

It was ascertained furthermore that a significantly higher variance of specific activities exists in the cranial part of the vertebra as compared with the trabecular bone in the distal femoral epiphysis. This could be in relation to osteosarcomas predominantly localized in the lumbar spine as reported by FINKEL et coll. (1969) and KLENER et coll. (1973) in experiments corresponding to the level of ^{226}Ra activity injected in the present experiment.

It is perhaps difficult to explain the difference in the specific activity with the same type of bone but in different parts of the skeleton. NILSSON (1969) reported on his experiments in mice after the administration of different ^{90}Sr levels. The incidence of osteosarcomas was stated to move from the axial skeleton into the long bones in relation to the decreasing dose of radionuclide injected. In the following discussion MAYS et coll. suggested that this might mainly depend upon the amount of the activity applied. It is perhaps not negligible which element from the series of alkali earths is applied, as follows from the kinetic investigations of ELLSASSER et coll. (1969) and LINIECKI (1971). Even the type and age of the animals ought to be considered because the differences in the process of ossification and the rate of remodelling of the bone tissue may play an important role (LOUTIT & VAUGHAN 1971).

Closely connected with this lies the metabolic activity of the cellular component

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Books received

We acknowledge with thanks under this heading books received for review; we trust this will be regarded as a sufficient mark of appreciation of the courtesy of the sender. Reviews of selected items will appear as soon as an opportunity affords.

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ACTA RADIOLOGICA

OFFICIAL ORGAN OF THE RADIOLOGICAL SOCIETIES OF DENMARK, FINLAND NORWAY AND SWEDEN

Vol 12 Fasc 5 | THERAPY PHYSICS BIOLOGY | 1973 October

TREATMENT OF 443 CASES OF SKIN CARCINOMA WITH CURETTAGE AND SOFT ROENTGEN RAYS BY THE EBBEHØJ METHOD

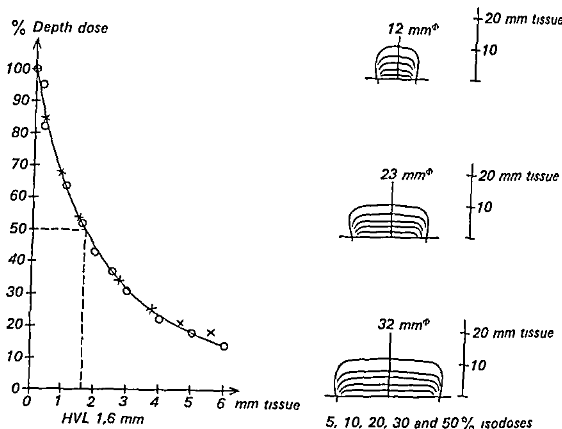
T Skov Jensen and M O Vetner

A special form of therapy for skin carcinoma with soft roentgen rays in a single dose, the so-called single session treatment, has been in use since 1935 in Denmark, where the method was introduced and systematized by Ebbehøj at the Radium Centre, Aarhus The results have been described earlier by Ebbehøj (1951) and Mosekilde (1951)

The purpose of this paper is to discuss the range of indication for employing this simple method, based upon an analysis of a series of 443 cases treated during the period 1948 to 1950

The Ebbehøj *method* The single session treatment by the Ebbehøj method is a combination of radiation therapy and surgery The whole tumour is first scraped thoroughly with a sharp curet until the treatment surface is flat and level and immediately afterwards, following the arrest of any bleeding, short distance roentgen irradiation is given at one session, the total amount thus being applied in a single dose

From the Radium Centre for Jutland (Director S Kaae), 8000 Aarhus Denmark
Submitted for publication 2 May 1972



Depth dose and isodose curves for 26 kV. HVL 0.1 mm Al. FSD 10 cm.

EBBEHØJ, in developing the method, used a roentgen apparatus for short distance therapy with a voltage of 26 kV, an FSD of 10 cm, and a tube with a filter of 0.2 mm Al (HVL to skin 1.6 mm). Basal cell carcinoma was treated with a total dose (air dose) of 4 200 R and prickle cell carcinoma with 5 000 R. The irradiated field included the primary tumour and a surrounding safety zone of 5 to 6 mm from the macroscopically visible margins of the growth. These principles for treatment have been applied throughout the period covered by the present investigation.

Depth dose and isodose curves for the 26 kV apparatus are given in the Figure. The depth dose measurements carried out by EBBEHØJ in 1937 with 0.2 mm paraffin plates revealed good agreement with measurements made in 1965 with film dosimetry; for the latter measurements the isodose curves were determined for three different tube types with diameters of 12 mm, 23 mm and 32 mm and recorded for 5, 10, 20, 30 and 50 per cent isodoses.

Table 1

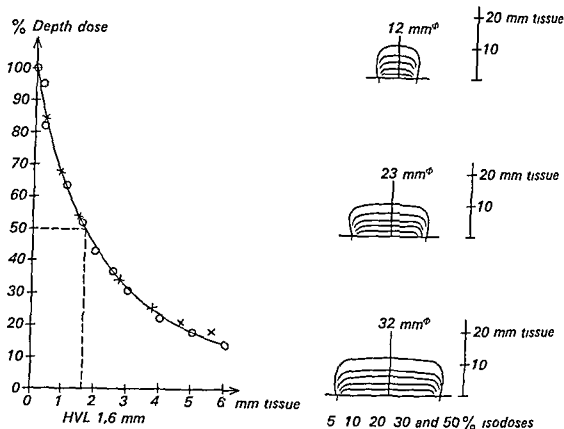
Results after single dose treatment in 443 patients with carcinoma of the skin

	Site	No of tumours	Freedom from recurrence after single dose therapy 1	2	3	4	1–4	Recurrences after one or several single dose treatments
Five year observation period	Nose	71	58	6	2		66 (93 %)	5
	Ear	32	28	3			31 (96 9 %)	1
	Palpebrae	33	29	2			31 (93 9 %)	2
	Other parts of face	177	156	10		1	167 (94 4 %)	10
	Body and extremities	41	35	5			40 (97 6 %)	1
	Total	354	306	26	2	1	335 (94 6 %)	19
five years	Nose	13	13				13	
	Ear	8	5	2			7	1
	Palpebrae	8	6	1			7	1
	Other parts of face	51	49	2			51	
	Body and extremities	9	9				9	
	Total	89	82	5			87 (97 8 %)	2

Present material A total of 561 dermal carcinomas were treated during the period 1948 to 1950, the external genital surfaces, the anal region and the prolabium of the lips are not included in this figure Of these 561 tumours, 489 had been given single dose treatment The histologic diagnosis was uncertain in 43 while 3 patients were not followed up after the primary therapy These 46 patients were therefore excluded from the material, 443 patients were thus available for the investigation

The other 72 tumours had been treated differently In some of these it was a question of a greater surface involvement or of deeper infiltration indicating the immediate use of fractionated short wave (50 to 70 kV) irradiation (30 tumours), others had received insufficiently radical surgical excision by radiation therapy (42 tumours) as a rule in fractionated doses

The 443 patients receiving single dose therapy and followed up were in agreement with other Danish materials as regards age, sex and histologic distribution (HANSEN & JENSEN 1968), 323 (73 %) of the patients were over 60, with a ratio of men to women of 3 2 The carcinomas were basocellular in 268 and of the prickle cell type in 160 patients, 15 growths contained both basal cell and prickle cell elements (mixed carcinoma)



Depth dose and isodose curves for 26 kV. HVL 0.1 mm Al. FSD 10 cm.

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The neoplasm was situated on the nose, ears or eyelids in 165 patients The frequency of recurrences after single dose therapy appears in Table 1

Among 55 recurrences, a cure was achieved after a second single dose treatment in 31, after 2 further treatments in 2, and after 3 further treatments in one patient The recurrences always lay in the marginal zone and no necrotic changes due to the radiation, or disfiguring scar tissue, subsequently arose The recurrences developing after the primary single therapy or after repeats required treating differently in the remaining 21 patients Fourteen of these were given surgical treatment and healing and freedom from recurrence was achieved in all, the operation was performed after the first recurrence in 7 of them

Irradiation with a larger HVL was tried in 4 patients Two of these had carcinoma of the limbus palpebrae (see the following section) and in a third there was a 20 mm × 25 mm malignant process at the apex of the nose encroaching on the vestibule The single session therapy was ineffective (a result that ought to have been foreseen), and after a time the process invaded the nasal septum As the patient would not consent to operation harder radiation qualities and electrocoagulation therapy were tried on several occasions, but death occurred within 10 years as the result of a large, ulcerative malignant process In the fourth patient with a deeply infiltrating dermal carcinoma of the forehead, a permanent recurrence developed after attempts with single dose treatments, this patient, who also refused operation, died 4 years later from another cause but with the recurrence still persisting

One patient died three months after 4 single session treatments, no information was available regarding neoplastic remnants The recurrences were associated with regional lymph node metastases (see a later section) in a further 2 patients.

Recurrences on ears and eyelids Malignancy was present in the external ear in 40 patients and a recurrence developed in 7 of them, healing was achieved in 5 of these after a repeat one session treatment. Partial ablation had to be undertaken in one patient in whom the recurrence developed three years after the first treatment In another patient, in whom single dose therapy had been given a second time for a clinical but not histologically confirmed marginal recurrence four months after the first treatment, rapidly growing regional lymph node metastases developed shortly afterwards

Forty-one tumours were located in the eyelids In 3 of these patients the growth had infiltrated both the anterior and posterior edges of the limbus palpebrae, and a relapse occurred soon after the single dose [illegible]
attempt [illegible]
suppleme[illegible]
however [illegible] tumours Freedom from recurrence was achieved after operation in the third patient

Table 2

Frequency of recurrence in relation to surface size of tumour

Greatest surface size of tumour	No of tumours			No of recurrences		
	Obs > 5 yrs	Obs < 5 yrs	Total	Obs > 5 yrs	Obs < 5 yrs	Total
< 10 mm	172	27	199	16	1	17 (8 5 %)
11—20 mm	149	42	191	21	3	24 (12 5 %)
21—30 mm	26	15	41	7	1	8 (19 5 %)
> 30 mm	7	5	12	4	2	6 (50 %)
Total	354	89	443	48	7	55

Results

The patients were controlled at regular intervals, usually 2, 6 and 12 months after treatment and subsequently at intervals of one year, the observation period in 354 patients was 5 years Primary healing of the tumour and a 5-year period with freedom from recurrence was achieved in 306 (86 5 per cent) of these patients (cf Table 1) Seventy patients in this group were under observation for at least 10 years and a late marginal recurrence occurred in the ninth year in 2 instances, in both of which cure was achieved by another single session

Eighty-nine patients were under observation for less than 5 years—23 for under 2 years, 21 for 2 to 3 years, 20 for 3 to 4 years, and 25 patients for 4 to 5 years—in whom 3, 1, 1 and 2 recurrences, respectively, developed

Local recurrences Development and treatment Recurrences within the course of the first 5 years arose in 55 of the 443 tumours, 25 of the relapses (46 per cent) were diagnosed within a half to one year after treatment while 15 (27 per cent) occurred at varying times in the first 5-year period The recurrence almost always developed, in 50 of 55 patients (91 per cent) in the marginal zone of the treatment field It was in the central area in 4 patients (7 per cent) and both at the centre and the periphery in 1 patient The latter had a superficial skin carcinoma with a surface size of about 7 cm² on the cheek, after repeat treatment with a single dose the patient had no further recurrence (5 years control) The risk of recurrence increases with the size of the primary condition, in Table 2 the frequency of recurrence has been related to the largest surface size With tumours measuring 21 mm to 30 mm at the most, the frequency is nearly 20 per cent, while for those over 30 mm it is approximately 50 per cent

words a slightly better result EBBEHØJ also noted a high proportion of cures after single dose treatments in dermal carcinoma A material from 1933 to 1936 of 85 patients produced 5 year freedom from recurrence in 83 instances (98 6 per cent)

This discrepancy in the treatment results is probably to be explained by the fact that the range of indications for a treatment that is so easy to administer may tend to be widened beyond the fairly narrow limits that have been set for the use of this method and that should be strictly observed

The results achieved with the method correspond approximately to those observed when other methods have been applied JOHANSEN (1961) observed a 5 year freedom from recurrence in 96 per cent of 334 patients with carcinoma of the skin when fractionated radiation treatment, mostly with 60 kV, was used MAGNUSSON (1935) mainly with brachyradium therapy obtained a 3-year freedom from recurrence in 91 to 93 per cent of patients with small superficial tumours MILASOV (1960) who employed electroresection in 298 small dermal carcinomas (< 2 cm) obtained freedom from recurrence over a 5 to 10 year period in 98 3 per cent and KNOX et coll (1967) also had the same good results with curettage and electrodesiccation In the two last materials, however, the ele [illegible] om surgical [illegible] methods in

[illegible] present material indicated that the marginal zone of the treatment field has so far been the commonest site for recurrences Probably, the main explanation of this is that it is often hard to assess the borders of a carcinoma of the skin with exactitude and that a safety margin of 5 to 6 mm beyond its periphery is not always achieved

Small marginal recurrences may usually be treated again with single dose therapy without any great risk of radiation necrosis or disfiguring scar formation Of 50 recurrences in the marginal zone in the present material, repeat single session treatments resulted in a cure in 34 patients (68 per cent) Repeated use of this therapy for recurrences should not be overdone, however A recurrence that has developed after correctly performed single dose treatment should as a general rule be treated surgically The relatively frequent use of repeat single dose treatments for recurrences in the present material suggests that the enthusiasm for the method has not always been in proportion to its possibilities Furthermore, it is sometimes to be wondered whether the chance of effecting a cure with the relevant primary treatment was not missed through delay owing to the use of the single dose therapy

Dermal neoplasms with a surface area of, at the most, 20 mm to 25 mm, should only exceptionally be treated with single dose therapy, and then only

The tumour had in 11 patient infiltrated only the anterior margin of the limbus palpebrae, and healing was always achieved after the first one session treatment. Ectropion and epiphora developed in 3 of these patients as a result of cicatricial scar formation; these complications were fairly mild, however, and did not require treatment.

Regional lymph node metastases. Development and treatment. Among the 443 skin carcinomas given single dose therapy were 206 prickle cell carcinomas, and regional lymph node metastases were established in 4 of them (2 per cent). The primary tumour had been located in the external ear in 3 of the latter and in the temporal region in the fourth. The regional lymph node metastases in these 4 patients developed within the first six months after the primary treatment. Lymph node dissection was carried out in 2 of them and external irradiation was given in the other 2 patients; they all died however within a few months with signs of further metastases. In 2 of these, both with the primary tumour in the external ear, this had healed before the metastases appeared.

Discussion

The problems connected with the treatment of small superficial carcinomas differ considerably from those in other forms of neoplasms. Malignancy of the skin seldom threatens the patient's life, and a cure can be achieved in several different ways, both by radiation therapy and by surgery. A form of treatment that may be given as an ambulant measure and at one sitting, and that may with a fairly high degree of certainty lead to a good result, offers considerable advantages to this patient group, in which the average age is high.

EBBEHØJ's method, with thorough scraping of the tumor and irradiation with soft roentgen rays administered in a single dose fulfils several of these requirements.

The present material comprised 354 tumours that were treated after careful scraping with 26 kV, 4 200 to 5 000 R, in a single dose, and freedom from recurrence was achieved in 86.5 per cent over a 5-year observation period. After supplementary one session treatments for small marginal recurrences that developed as a rule within the first half to one year after the primary therapy, the figure for freedom from recurrence was 94.6 per cent.

A corresponding treatment series from the same department comprising 420 tumours treated in the same way with a single dose during the period 1937 to 1942 indicated, according to MOSEKILDE (1951), freedom from recurrence in 91.4 per cent and 97.1 per cent respectively, after 5 years observation, in other

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A corresponding treatment series from the same department comprising 420 tumours treated in the same way with a single dose during the period 1937 to 1942 indicated, according to MOSEKILDE (1951), freedom from recurrence in 91.4 per cent and 97.1 per cent respectively, after 5 years observation, in other

SUMMARY

The range of indications for the use of single dose therapy in carcinoma of the skin has been based on the results obtained in 443 patients by scraping the lesion and irradiating it with soft roentgen rays in a single dose. The percentage of cures with one or possibly several treatments of small superficial tumours was 95 to 97 % over a 5 year observation period. The method is easy and quick and free from complications.

ZUSAMMENFASSUNG

Der Indikationsbereich für die Anwendung einer Einzeldosistherapie beim Hautkarzinom stützt sich auf die Ergebnisse von 443 Patienten, bei denen die Veränderungen abgekratzt wurden und mit weichen Röntgenstrahlen in einer Einzeldosis bestrahlt wurden. Die Heilungsrate mit einer oder möglicherweise mehreren Behandlungen von kleinen oberflächlichen Tumoren betrug während einer 5 jährigen Beobachtungszeit 95—97 %. Die Methode ist einfach, rasch und komplikationsfrei.

RÉSUMÉ

Le domaine des indications pour le traitement par une dose unique dans le cancer de la peau a été établi d'après les résultats obtenus sur 443 malades par raclage de la lésion et irradiation par des rayons roentgen mous en une dose unique. Le pourcentage de guérison avec un ou éventuellement plusieurs traitements de petites tumeurs superficielles a été de 95 à 97 % sur une période d'observation de 5 ans. Cette méthode est facile et rapide et exempte de complications.

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when the growth is more or less superficial. If this treatment is applied to larger tumours or deeply infiltrating growths the risk of recurrence is high.

Tumours situated in the ears, nose or eyelids presented the same degree of healing *in this material* as those at other sites. Single dose treatment had, however, been given in only 4 patients when the neoplasm had a surface larger than 20 mm, and a *recurrence developed in 3 of these. Neoplasms involving the* limbus palpebrae, the cartilage of the ear or the nasal septum, or situated on irregular surfaces that cannot be levelled are *not suitable for single dose therapy.*

As roughly 90 per cent of all skin carcinomas occur on the face, consideration should be paid to the cosmetic aspect when the form of therapy is to be selected, even though the average age of the patients is relatively high. The cosmetic result is usually acceptable with the LEBBEHOJ one session treatment. The radiation scar is generally only moderately conspicuous, with only moderate depigmentation and epidermal atrophy. A hyperpigmented marginal zone often develops during the first months following the treatment, but this gradually disappears. Only in larger and more deeply infiltrating carcinomas is the scar more obvious, with excavation and cicatricial shrinking. Where cartilaginous tissue lies at the surface (in the external ear, for instance), a risk also exists that chondritis will *arise with single dose treatment. This occurred in 3 of the 40 patients in the* present material who had neoplasms of the external ear, but they disappeared spontaneously within 3 to 12 months.

Conclusions

Single dose treatment of carcinoma of *the skin* by the EBBEHOJ method may be applied if the following requirements have been met:

1) *The surface area of the tumour should not exceed 20 mm and the infiltra*tion should not be deeper than 1 to 2 mm.

2) The treatment surface should be level.

3) The whole tumour should be scraped thoroughly by sharp curettage.

4) The treatment field should be of such a size that the distance from the edge of the field to the periphery of the growth is 5 to 6 mm.

5) Tumours located in the external ear (for instance in the cavum or cymba conchae), in the surrounding parts of the eye with infiltration of the limbus palpebrae, or on irregular surfaces such as the ala nasi or the apex of the nose, should be treated by the single dose method *only if their surface area measures* less than 10 mm. Primary operative treatment is often indicated instead.

6) The treatment may be repeated for small marginal recurrences.

7) The cosmetic result may be considered acceptable.

Table 1

Mandibular bone involvement Sex and age distribution

Age	Male	Female	Total
30 – 39	1	0	1
40 – 49	5	2	7
50 – 59	15	0	15
60 – 69	13	4	17
70 – 79	9	3	12
80 – 89	2	0	2
90 – 99	0	1	1
Total	45	10	55

Table 2

Mandibular bone involvement Site of origin of the primary lesion

Site	No of patients	
Floor of the mouth	8	(14 %)
Lower alveolus	29	(53 %)
Tonsillar region	17	(31 %)
Mouth site undetermінate	1	(2 %)
Total	55	(100 %)

Table 3

Mandibular bone involvement Distribution of lesions by stages

	No of patients	
Stage I	1	(2 %)
Stage II	35	(64 %)
Stage III	19	(34 %)
Total	55	(100 %)

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CARCINOMA OF THE MANDIBLE

Result of radiation therapy

JUAN V FAYOS

In the orthovoltage radiation era, the presence of bone involvement by squamous cell carcinoma of the oral cavity was usually considered an indication for surgery This was due to the inability to give large doses of radiation without producing severe damage to the vasculo-connective tissue of the jaw bone which eventually could progress into a full-fledged necrosis of bone Lower doses of radiation were incapable of controlling the primary tumor

With the advent of supervoltage irradiation, the soft tissues constituting the matrix of the bone did not receive such large doses of scattered radiation and therefore the ensuing damage to the vasculo-connective tissue was minimized, thus permitting a higher dose to the primary lesion at tolerable levels to the bone In this fashion it was possible to irradiate the primary lesion without producing significant damage to the bone irradiated

At the University of Michigan, we obtained supervoltage radiation in the year 1955, in the form of a Cobalt 60 unit Since that year to the end of 1969 we have completed treatment in 55 patients with involvement of the mandible to variable degrees This investigation is to report on the incidence of the mandibular involvement, its treatment, and complications, local results, actuarial survival and complications

Submitted for publication 2 October 1972



Fig ? a) Extensive lesion of the right lower alveolus without osseous involvement b) Post irradiation appearance of lower jaw Loss of alveolar ridge on the right (arrow)

Two per cent of the lesions were classified as stage I, 64 per cent were stage II and 34 per cent were stage III (Table 3) We classified as stage I those small primary lesions with no metastasis (T1, T2, N0, M0 cases) Stage II were those lesions with large primaries and no metastasis in the neck or with small primaries having early metastasis to the neck lymph nodes (T3, T4, N0, M0 and T1, T2, N1 M0 cases)

In stage III we recorded all of the remaining cases, some with neck metastasis of considerable extent In this stage the prognosis is always poor, reflecting the biologic effects of metastasizing neoplasm

Degree of bone involvement The degree of bone involvement was classified into three groups depending on the findings of pre irradiation films The lesion in one patient with an intra alveolar squamous cell carcinoma of the jaw was considered in this review as arising in the gum The cases classified as having *erosion* of bone (33 per cent), were those in which the cortex of the bone appeared depressed and eroded as if produced by tumor pressure (SWEARINGEN et coll 1966) The invasive group (49 per cent) had some mottled destruction of bone We included in this group not only those cases with massive destruction of bone, but also those in which the *elements of* lytic destruction were associated with elements of erosion of the cortex (Fig 1) Finally in 18 per cent of the cases, routine films of the mandible did not show bony abnormalities, due either to the incipient character of the destruction, to its position, or to poor bony details of the routine mandibular films However, in these cases there was unequivocal clinical evidence of destruction of bone, particularly the alveolar



a



b



c

Fig 1 a) Tumor of lower alveolus b) Pressure and lytic destruction of bone c) The healed post irradiation status

Material

Age and sex Table 1 shows the distribution of patients according to sex and age in cases treated from 1955 through 1969 The mean age is 62 with a standard deviation of 12 years

Distribution of cases The sites of origin of the primary lesion are shown in Table 2 The most common primary lesion originated in the mucosa of the lower alveolus They constituted about half of the carcinomas that involved the jaw Next in frequency were lesions arising in the tonsillar region, mainly in the retro molar triangle It is common for this type of tumor to grow posteriorly and inferiorly and to involve the adjacent bone



a b

Fig 3 a) Carcinoma of lower alveolar mucosa with bony erosion b) Primary lesion controlled with radiation therapy Uncomplicated healing of the neoplastic bony defect

year survival was obtained (Table 4) The relative survival (ratio of observed to expected survival) was 31 per cent at 5 years

If we correlate the survival of patients and their stage, we find that the patient in stage I died of the disease Of the 35 patients in stage II, 7 were alive at 5 or more years, 3 alive less than 5 years, 18 died of the neoplasm and 7 died before 5 years of an intercurrent death with no evidence of disease at the time of death Of the 19 patients in stage III, 4 were alive more than 5 years, 1 was alive without disease less than 1 year, 12 died of the disease and 2 died an intercurrent death

In order to determine the effectiveness of radiation as a curative agent for carcinomas involving the mandible, we ascertained in each case whether radiation therapy did or did not control the primary lesion By control we mean that the primary lesion did not show evidence of recurrence at any time after treatment *until* the last follow up If the patient died of intercurrent disease, the minimal post irradiation period was one year If the patient with controlled tumor died within one year the case was placed with the unknown group together with the ones in which complete information was not available Not controlled were those cases that at any time in the post irradiation period developed a recurrence, strictly, these are failures of radiation therapy Th

Table 4

Mandibular bone involvement: Actuarial survival

Year interval	Alive at beginning of interval	Dead of neoplasm during interval	Died of intercurrent disease during interval	Withdrawn alive during interval	Cumulative proportion surviving at end of interval	
					[illegible]*	Relative survival**
0–1	55	16	1	0	0.71	0.72
1–2	28	9	2	1	0.53	0.53
2–3	26	2	1	0	0.49	0.49
3–4	23	4	2	0	0.40	0.37
4–5	17	0	3	3	0.40	0.34

* Dead of intercurrent disease considered as withdrawn alive at that interval

** Observed survival/expected survival

crest. The initial clinical impression of involvement of bone was reinforced by the appearance of the mandible in the post irradiation period, a mandibular defect at the site of the previous tumor (Fig. 2). Currently the routine mandibular films are supplemented when indicated by Panorex- and occlusal dental films.

Treatment

Invariably the patients were treated with Cobalt 60 radiation at a source skin distance of 73 to 80 cm. Most often the lesion was irradiated using opposing cervico-facial fields that encompassed the primary lesion and the neck metastasis if present. Both fields were treated on a daily basis, in the early years 6 days a week and more recently 5 days. The daily tumor dose was 200 R or slightly larger for a total dose of about 6 500 R given in a period of 6 to 7 weeks.

Results

For the calculation of survival we have utilized the actuarial method of calculation. Results from 1 to 5 years are presented taking into consideration whether the patients who died of an intercurrent disease were withdrawn alive or not at the interval in which the event occurred, in this fashion a 40 per cent 5-



a b

Fig 3 a) Carcinoma of lower alveolar mucosa with bony erosion b) Primary lesion controlled with radiation therapy Uncomplicated healing of the neoplastic bony defect

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It is of interest that some patients died of advancing neoplasm either in the neck or beyond while their primary lesion was controlled In 9 patients, recurrence after a complete course of irradiation was treated surgically In one a small residuum was successfully excised locally without recourse to further surgery (this patient was alive 12 years after initial treatment) In the rest, more extensive surgery was employed and usually consisted of resection of the residual tumor, hemimandibulectomy, and radical neck dissection In none of the cases were there any post-operative complications attributable to previous irradiation The mucosa healed over the resected bone without apparent difficulties

Of the 9 patients that had post-irradiation surgery, 6 were alive (4 after 5 years and 2 less than 5 years) and 3 died of the disease with an uncontrolled primary

Complications

In all cases of controlled primary the mucosa of the area involved healed without difficulty (Fig 3)

One patient developed focal exposure of the jaw in the post-irradiation period This sequestrum was eliminated spontaneously and the mucosa healed over the area of necrosis without further complications Another patient had necrosis of the soft tissues of the mouth but healed spontaneously without sequelae In one patient marked induration of the subcutaneous tissues of the face developed several years after irradiation This was due to the technique of treatment employed only an ipsilateral field to an incidence dose of 7 250 R was used No instance of progressive necrosis of the jaw was found

Discussions

The concept that irradiation is not indicated whenever there is involvement of bone by squamous cell carcinoma is no longer tenable if supervoltage radiation is used (CATANTA et coll 1970, PORTER 1971) In 55 cases with involvement of the jaw in patients treated from 1955 through 1969, the local control of the lesion was 36 per cent In the 9 patients that had an uncontrolled primary and were operable, 6 had control of the primary lesion after post-irradiation surgery The other 3 patients died of the disease with uncontrolled primaries It is worth emphazising that no patient having post-irradiation surgery developed bone complications, and healing of the mucosa and skin proceeded without apparent difficulty

The actuarial relative survival at 5 years is 40 per cent This figure was obtained counting as alive patients salvaged by post-irradiation surgery Most of the lesions treated were in intermediate and advance stages, 64 per cent in stage II and 34 per cent in stage III In the later stage, the prognosis is ominous in view of the advanced nature of the disease at the primary and metastatic sites, yet a certain number of patients in this stage were cured of the neoplasm (of 19 stage III patients, 4 were alive more than 5 years and 2 died an intercurrent death, the rest died of the disease)

From the findings of this investigation we would advocate continuation of the present program of supervoltage irradiation of the squamous cell carcinoma infiltrating bone, followed by radical surgery if a radiation failure becomes evident in the post irradiation period

The number of complications following irradiation was low Only one patient developed exposure of mandibular bone, with the exposed area healing after removal of the sequestrum Another patient developed marked induration of the subcutaneous tissues of the face This patient was treated using an ipsilateral field with a high incident dose This technique, although rarely used, was abandoned Instead, two opposing lateral fields are used, each field treated daily, 5 or 6 times a week In this fashion we believe that the radiation damage to the vasculo-connective tissue is diminished due to a more homogenous irradiation of the area treated

SUMMARY

Fifty five patients with squamous cell carcinoma involving the mandible were treated with *Cobalt 60* irradiation A 5 year actuarial survival of 40 per cent was found Nine patients had post *irradiation* surgery for recurrence, 6 had control of the neoplasm No post operative complications were *recorded* The rate of radiation complications was low Only one patient developed exposure of bone, which healed spontaneously We advocate super voltage radiation *therapy to squamous* cell carcinoma involving the mandibular bone, followed by radical surgery only if tumour remnants or recurrence become evident in the post irradiation period

ZUSAMMENFASSUNG

Funfundfunfzig Patienten mit einem Schuppenzellkarzinom unter Beteiligung des Unter kiefers wurden mit Cobalt-60-*Bestrahlung* behandelt Es wurde eine 5 Jahre Überlebensrate von 40 % gefunden Neun Patienten wurden wegen eines Rezidivs nach der Bestrahlung chirurgisch behandelt bei 6 war das Neoplasma unter Kontrolle Es wurden keine post operative Komplikationen gefunden Die Frequenz von Strahlenkomplikationen war niedrig Bei nur einem Patienten entwickelte *sich* eine Freilegung des Knochens welche spontan abheilte Wir befurworten Hochvolt Strahlentherapie von Schuppenzellkarzinomen *unter* Beteiligung des Unterkiefer *Knochens* mit nachfolgender radikaler Chirurgie nur wenn der Tumor noch vorhanden ist oder wenn in der Nachbestrahlungs Perioden ein Rezidiv auftritt

RÉSUMÉ

Cinquante-cinq malades présentant un carcinome épidermoide atteignant la mandibule ont été traités par les radiations au Cobalt 60. La survie actuarielle à 5 ans a été 40%. Neuf malades ont subi une intervention chirurgicale après l'irradiation pour une récidive, 6 malades étaient guéris. On n'a pas constaté de complications post-operatoires. La fréquence des complications due aux radiations a été faible. L'os n'a été mis à nu que chez un seule malade qui a guéri spontanément. Nous recommandons le traitement par les radiations de haute énergie pour les carcinomes épidermoides intéressant l'os mandibulaire, suivi de chirurgie radicale seulement si les restes de tumeur ou une récidive se manifestent dans la période après l'irradiation.

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DIAGNOSTIC SCORING SYSTEM FOR MAMMARY CARCINOMA

J W BIRSNER and J GERSHON COHEN

The sensitivity and specificity of such diagnostic measures as physical examination, roentgen examination of the breast and thermography were recently appraised in 862 patients with mammary carcinoma in six large medical centers This was a conjoint effort under the auspices of the American Cancer Society, and the results were analyzed by expert statisticians All three disciplines were found to have approximately the same degree of diagnostic accuracy (LILIENFELD et coll 1969)

One of the present authors (J W B), when discussing deficiencies in the discovery of early mammary carcinoma, proposed the evolution of a simple point system, which would assign appropriate weights to the various diagnostic modalities contributing to better clinical judgement Once the system was tentatively established, the other author (J G-C) agreed to test it retrospectively

This is a preliminary report of the efforts to convert the diagnosis of breast diseases from an intuitive or analogue method to a more precise or digitized discipline The diagnostic items used in the scoring were the following (1) the patient's age, (2) duration of symptoms, (3) history of mammary carcinoma

Submitted for publication 4 June 1971

Table 1

Age factor points in the incidence of mammary carcinoma

Age	Points assigned
30–34	5
36–39	15
40–44	30
45–49	40
50–54	40
55–59	40
60–64	35
65–69	35
70–74	35
75–79	35
80 84	45
85–89	60

in (a) mother or sister, (b) mother and sister, (c) aunt, (4) previous breast surgery, including aspiration of cysts and mastectomy, (5) physical examination, (6) roentgen findings, and (7) thermographic findings

Age factors Since the incidence of mammary carcinoma increases commensurate with increasing age, except for a slight plateau just after the menopause, points varying with established carcinoma incidence were assigned each decade, beginning with the fourth (Table 1) The points assigned to progressive aging are heavily weighted diagnostically because this factor is so well documented (DUNN 1969)

Duration of symptoms Ten points are given for symptoms present less than two months and five points for symptoms present more than two months, on the theory that benign lesions are more apt to be associated with chronicity than malignant ones (Table 2)

Family history If there is a history of mammary carcinoma in a sister, a mother or an aunt, 10 points are assigned If carcinoma has occurred in both a sister and a mother, 15 points are assigned (Table 2)

Previous breast surgery Patients who have had aspiration of a cyst or other breast surgery are assigned 10 points for the first operation and 5 more points

Table 2

Diagnostic scoring of symptoms, family history and breast surgery

	Points assigned
Symptoms, duration	
Less than 2 months	10
More than 2 months	5
Mammary carcinoma in family	
In sister mother, aunt	10
In sister and mother	15
Breast surgery	
One operation	10
Two operations	15
Three operations	20
Mastectomy	25

are added for each subsequent surgical maneuver. Chronic cystic mastitis (mazoplastica cystica) is usually the basis for aspiration of cysts, and these points are assigned because carcinoma is said to occur more frequently in these circumstances If one breast has already been removed for carcinoma, 25 points are scored, since such patients may be considered to be in a higher risk category (Table 2)

Physical examination Values for the components of the physical examination are shown in Table 3 If the examination reveals nothing abnormal, no points are assigned If the findings are indefinite or inconclusive, 10 points are given. The same points are given to any nipple discharge or bleeding A lump which is movable and 'feels benign' is allotted 20 points A 'dominant mass' that is fixed or has the classical signs of malignancy is credited with 50 points This is further substantiated if the recorded diameter of the mass is greater clinically than on the roentgen film Eczema in or around the nipple also calls for 50 points because of the possibility of Paget's disease Many more sophisticated physical findings considered to contribute to the 'high risk' classification might be incorporated into the scoring by experienced clinicians

Roentgen examination The points assigned to various aspects of soft tissue examination are shown in Table 4 If the breasts appear to be healthy, no points are assigned Should dysplasias be detected, no matter what the type, 5 points are

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70–74	35
75–79	35
80–84	45
85–89	60

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Table 3

Scoring of physical examination

	Points assigned
Negative findings	0
Indeterminate findings, including nipple discharge	10
Mass, movable, 'benign'	20
Mass fixed dominant, 'malignant'	50
Eczema of nipple	50

scored if the involvement is minimal, 10 points if it is moderate, and 15 points if it is severe. If a breast is encountered that is either excessively dysplastic or atrophic in respect to the patient's chronologic age, 30 points are tabulated for this 'disproportion', on the premise we entertain that carcinoma develops more frequently in such breasts. If the dysplasia is due to epithelial hyperplasia or sclerosing andenosis, 30 points are warranted, for such breasts seem to have a greater incidence of malignancy than do those associated with the other dysplasias, most of which are due to mild or benign physiologic aberrations.

If 'indirect' signs of malignancy are found on the films, such as the presence of a smoothly contoured but solitary or isolated tumor, especially if its diameter is less than that recorded clinically, or if there is distortion of the morphologic appearance, especially if accompanied by edema and skin thickening, 50 points are scored. For the 'direct' signs, such as a spiculated mass which is obviously a scirrhous carcinoma, or aggregates of microcalcifications that are pathogno-

Table 4

Scoring of roentgen examination of the breast

	Points assigned
Normal breasts	0
Dysplasias	
Minimal	5
Moderate	10
Severe	15
Disproportion between biologic and chronologic age	30
Carcinoma: indirect signs	
Distorted morphology, isolated smooth mass, edema, skin thickening	50
Carcinoma: direct signs	
Spiculated mass, microcalcifications	100

Table 5

Scoring of thermographic findings

	Points assigned
Normal appearance	0
Increase of temperature more than 0 5 C whether localized diffuse or venous	30

monic of carcinoma, 100 points are allotted, sufficient to refer the case automatically into the category of malignancy

There are those who feel that the presence of axillary lymph nodes more than 1 cm in diameter should be given some consideration when found on the films It is true that such nodes may be due to carcinoma (ALEXANDER & HALL 1970, GERSHON COHEN & HERMEL 1969) However, enlarged non-solid nodes are frequently associated with dysplasias, metastatic nodes appear to be solid because of blocking and packing of the medullary centers with abundant mitotic cells We have not used scores for this finding because we do not yet have confidence in the security of these observations Many radiologists might want to consider different plans of scoring, greater or lesser in scope, depending on their own experience Such variations are to be encouraged if they prove helpful

Thermography For those having access to thermography, the scoring of this examination is detailed in Table 5 Where thermography is not available, the scoring system could be appropriately altered

Table 6

Zone scoring of points among healthy women and those with benign and malignant lesions

Score	Normal			Benign			Malignant		
	No of cases	Per cent	Cumulative per cent	No of cases	Per cent	Cumulative per cent	No of cases	Per cent	Cumulative per cent
0 25	3	3 0	3 0	0	0	0	0	0	0
30–75	58	58 0	61 0	10	31 0	31 0	0	0	0
80 105	31	31 0	92 0	10	31 0	62 0	15	23 0	23 0
110 140	8	8 0	100 0	6	19 0	81 0	13	19 5	42 5
145 165				6	19 0	100 0	17	26 5	69 0
165							20	31 0	100 0

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Carcinoma indirect signs	
Distorted morphology isolated smooth mass edema skin thickening	50
Carcinoma 'direct signs	
Spiculated mass microcalcifications	100

Table 5

Scoring of thermographic findings

	Points assigned
Normal appearance	0
Increase of temperature more than 0 5 C whether local ze l d ffuse or venous	30

monic of carcinoma, 100 points are allotted, sufficient to refer the case automatically into the category of malignancy

There are those who feel that the presence of axillary lymph nodes more than 1 cm in diameter should be given some consideration when found on the films It is true that such nodes may be due to carcinoma (ALEXANDER & HALL 1970, GERSHON COHEN & HERMEL 1969) However, enlarged non solid nodes are frequently associated with dysplasias, metastatic nodes appear to be solid because of blocking and packing of the medullary centers with abundant mitotic cells We have not used scores for this finding because we do not yet have confidence in the security of these observations Many radiologists might want to consider different plans of scoring, greater or lesser in scope, depending on their own experience Such variations are to be encouraged if they prove helpful

Thermography For those having access to thermography, the scoring of this examination is detailed in Table 5 Where thermography is not available, the scoring system could be appropriately altered

Table 6

Zone scoring of points among healthy women and those with benign and malignant lesions

Score	Normal			Benign			Malignant		
	No of cases	Per cent	Cumulative per cent	No of cases	Per cent	Cumulative per cent	No of cases	Per cent	Cumulative per cent
0 25	3	3 0	3 0	0	0	0	0	0	0
30 75	58	58 0	61 0	10	31 0	31 0	0	0	0
80 105	31	31 0	92 0	10	31 0	62 0	15	23 0	23 0
110 140	8	8 0	100 0	6	19 0	81 0	13	19 5	42 5
145 165				6	19 0	100 0	17	26 5	69 0
165							20	31 0	100 0

Table 3

Scoring of physical examination

	Points assigned
Negative findings	0
Indeterminate findings, including nipple discharge	10
Mass movable, 'benign'	20
Mass, fixed dominant, 'malignant'	50
Eczema of nipple	50

scored if the involvement is minimal, 10 points if it is moderate, and 15 points if it is severe. If a breast is encountered that is either excessively dysplastic or atrophic in respect to the patient's chronologic age, 30 points are tabulated for this 'disproportion', on the premise we entertain that carcinoma develops more frequently in such breasts. If the dysplasia is due to epithelial hyperplasia or sclerosing andenosis, 30 points are warranted, for such breasts seem to have a greater incidence of malignancy than do those associated with the other dysplasias, most of which are due to mild or benign physiologic aberrations.

If 'indirect' signs of malignancy are found on the films, such as the presence of a smoothly contoured but solitary or isolated tumor, especially if its diameter is less than that recorded clinically, or if there is distortion of the morphologic appearance, especially if accompanied by edema and skin thickening, 50 points are scored. For the 'direct' signs, such as a spiculated mass which is obviously a scirrhous carcinoma, or aggregates of microcalcifications that are pathogno-

Table 4

Scoring of roentgen examination of the breast

	Points assigned
Normal breasts	0
Dysplasias	
Minimal	5
Moderate	10
Severe	15
Disproportion between biologic and chronologic age	30
Carcinoma, indirect signs	
Distorted morphology, isolated smooth mass, edema, skin thickening	50
Carcinoma, direct signs	
Spiculated mass, microcalcifications	100



Fig 1 Mammary radiography Adenocarcinoma (→) found in a 61-year-old patient who complained of only discomfort in the upper outer portion of the left breast for one month A movable tender mass was palpated and thought to be benign Its measurements were greater on the patient than on the film and it was scored according to our system with 50 points Other points accrued were age 35 symptoms 10 breast cancer in family 10 prior breast surgery 25 (aspiration of cyst 45) physical examination 20 thermography 30 Total points 180



Fig 2 Thermogram of patient [illegible] The red [illegible] of breast [illegible] areas 25 C [illegible] than the green [illegible] There are [illegible] changes between red and green each [illegible]

About 20 per cent of all women will have one or more localized signs of increased infrared emission, and this is also true of about 40 per cent of women with dysplasias (GERSHON-COHEN & HERMEL 1969, GERSHON-COHEN et coll 1970 a) But because some 90 per cent of women with malignancy also display such signs, the scoring for thermographic infrared radiation is placed at 30 points

Originally we insisted that the localized 'hot spot' be at least 1° C greater *than in the counterpart area of the opposite breast, but we no longer adhere to* this criterion In some proved carcinomas the difference in temperature was less than 0 5° C, and in others the increase in heat was diffuse rather than localized, or was confined to a segment of a superficial vein Moreover, we have frequently found that large temperature differences, such as between 4° C and 6° C, are just as apt to portend active dysplasias as carcinomas The thermographic scores can be very important In some 5 per cent of cases a positive thermogram was mainly responsible for the detection of malignancy, and we recently reported 4 cases illustrating this circumstance (GERSHON-COHEN et coll 1970 b)

Results

This scoring system has been tested retrospectively on patients selected from those examined during the last 24 months, when an advanced roentgen apparatus, the Senograph (manufactured in France, distributed in the United States by Keleket/CGR, Waltham, Massachusetts), was available This new equipment makes possible the use of an improved technique with distinctive advantages over conventional equipment, and has materially enhanced the *accuracy of the interpretation* (GERSHON-COHEN *et coll* 1970 c, GERSHON-COHEN 1970) Of about 1 450 patients examined during this period, pathologic reports are available in 97 cases, in 65 cases of carcinoma in 63 women (2 bilateral) and in 32 cases of lesions that proved to be benign As controls we selected and scored 100 patients of similar ages and symptomatology in whom malignancy was not suggested and who therefore were not subjected to biopsy The duration of follow-up in these 'normal' patients is less than two years, but in none has evidence of malignancy emerged thus far

Controls Of the 100 controls, it will be noted (Table 6) that 61 cases scored below 75 points In the zone between 80 and 105 points fell 31 cases, and the remaining 8 patients scored above 110 points Reviewing the scores of these 8 patients, we found that their ages ranged from 46 to 69 years, averaging 53 years These patients will be more frequently examined, since we regard them as 'high risk' patients

Benign lesions Of the 32 cases whose biopsies proved to be benign, it is noteworthy that the radiologist, without benefit of the point system, suggested the presence of malignancy in 12 instances In the other 20 cases, he suggested biopsy merely to confirm his impression of benignancy which he preferred to be checked by histology

In the 12 'false positives', retrospective analysis of 8 of them reveals a range from 50 to 155 points, with an average of 110 points In the other 4 cases, microcalcifications characteristic of carcinoma were present, and unless we are privileged to re-examine these patients to assure ourselves that the microcalcifications have been removed, our appraisal of them remains sub judice These cases scored from 140 to 165 points, with an average of 155 points

In the 20 cases of the total 32 in which biopsy was suggested to establish benignancy, the point system applied retrospectively shows a range of from 30 to 150 points (1 case), with an average of 82 points

Malignant lesions Our scoring techniques in a case of carcinoma is illustrated in Figures 1 and 2 Without benefit of this point system, it must be noted that in the original diagnosis of the 65 carcinomas, 10 were incorrectly diagnosed as negative Eight of these 10 false negative fell in the point range between 80 and 105 points

Retrospective review of these 8 cases shows that the patients ranged in age from 29 to 53 years averaging 43 years, with an average point score of 90 The difficulties of interpreting the films of the dysplastic breasts of younger women undoubtedly contributed to our failures It is possible that refinement of diagnostic accuracy might be achieved if Boolean clustering were used in this group (FEINSTEIN 1967) In the other 2 false negatives, retrospective analysis shows point scores of 145 and 230, high enough to prompt a diagnosis of malignancy

One of the most difficult features in the interpretation of the films is the *recognition* of distorted morphology caused by malignancy, usually of the invasive duct cell variety before the formation of a *scirrhus* Failure to note such distortions results in only a 10 point assessment, ± 5 points, whereas detection of distortion results in the allotment of 50 points, a critical difference In both these 2 false negatives, morphologic appearance had been overlooked

In our prospective tests, we are emphasizing special care in appraising cases with scores between 80 and 105 points Reference to Table 6 indicates that an appreciable percentage of patients with these scores are found to harbor carcinoma In the next zone, with points between 110 and 140, the percentage of women with malignancy climbs and those who have greater than 145 points almost uniformly are found to have mammary carcinoma

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Discussion

When auction bidding in the game of bridge was converted to a point system of contract bidding, it crystallized in the bidder's mind the full worth of his hand By analogy, we feel that by employing a scoring system, the diagnosis of mammary carcinoma can be approached more precisely For the time being, the purpose of this report is merely to introduce the concept Refinements, such as 'Boolean clustering', high-risk factors such as marital status, fertility, age at menarche and menopause, endocrine profiles, et cetera, can be added later Since our objective is to keep the system as simple as possible for the present, we elected to give point assignments only to the least controversial factors

If and when this point system is made firm and is adopted, an entirely new concept of the high risk patient could emerge Clinicians have generally limited this category to women with a family history of mammary carcinoma, or to patients who have had a mastectomy This narrow point of view ought to be expanded, and this could be accomplished by placing due weight upon all the factors that comprise an integrated medical consultation

The use of such a system would stimulate clinicians to make a thorough physical examination, with proper scoring of its various components The same tenet holds for the radiologist and the thermographer As medical computers come more and more into play, these are precisely the factors physicians will have to take into consideration for accurate programming

When a carcinoma has advanced far enough to produce the classic signs of induration, fixation, and the secondary signs of edema and lymph node metastasis, the point system admittely can do little more than confirm the malignancy It is not, however, the advanced but the early tumor, still localized and therefore difficult to detect clinically, that we hope may be snared by the use of this scoring system For example, when an isolated, circumscribed mass is detected which could be benign as well as malignant, leaving much ambiguity in the examiner's mind, the 20 points assigned to it by the physical examination and the 50 points assigned by soft tissue roentgen examination are usually enough to result in a total above 125 points, emphasizing the need for biopsy to decide the issue

Finally, we hesitate less to suggest biopsy in patients with scores between 80 and 140 points If malignancy is found, we are becoming more and more inclined to favor conservative therapy with reconstruction of the breast rather than mutilative radical mastectomy Other investigators have found that such procedurs offer results as good as any other form of therapy and can often be combined with radiation therapy (BADER et coll 1970, CRILE 1968)

In light of the difficulties pathologists have in locating minute areas of malignancy, as recently reported by ROSEN et coll (1970) and HUTTER et coll

(1969), the day may come when patients with points falling into the zone between 80 and 105 will automatically be subjected to reconstructive surgery. In such cases, the removed tissue could then be routinely examined roentgenographically so as to guide the pathologist in his selection of tissue for histologic examination. Only in this manner can the pathologist hope to detect the small carcinomas that in ordinary circumstances might be overlooked (BADER et coll 1970, ROSEN et coll 1970). Carcinoma in situ occurs far more frequently than is generally realized (FARROW 1970).

SUMMARY

Various modalities used to diagnose mammary carcinoma were analyzed and assigned diagnostic weights to provide a simple point scoring system. It is suggested that the introduction of such a system of diagnosis could lead to more accurate clinical judgment and be useful for computer programming.

ZUSAMMENFASSUNG

Verschiedene zur Diagnose eines Mammakarzinoms verwendete Verfahren wurden analysiert und deren diagnostischer Wert bestimmt, um ein einfaches Punkte-Rechensystem zu erhalten. Es wird die Vorstellung entwickelt, dass die Einführung eines solchen diagnostischen Systems zu einer genaueren klinischen Beurteilung führt und bei einer Computer-Programmierung nützlich ist.

RÉSUMÉ

Les auteurs ont étudié différentes techniques utilisées pour le diagnostic du cancer du sein et leur ont affecté un «poids» diagnostique pour établir un système simple de «calcul par point». Ils pensent que l'introduction d'un tel système de diagnostic pourrait conduire à un jugement clinique plus précis et pourrait être utile pour la programmation sur ordinateur.

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FROM THE RADIATION CENTER, LATTER DAY SAINTS HOSPITAL, SALT LAKE CITY, UTAH 84143, U S A

AN EASY WAY OF IMPROVING THE FLATNESS OF THE RADIATION FROM A LINEAR ACCELERATOR

W H FRY, R H HUERTA and M B SCHIRMER

Because of the predominantly forward emission of rays from the target of linear accelerators operating in the megavoltage region, it is essential that flattening filters be inserted in the beam in order to provide a therapeutically useful beam of radiation The degree of flattening necessary is, to some extent, a matter of opinion, but it would be sensible that the flatness should be at least as good as that of a Cobalt beam and preferably better The flattening filters are usually made of heavy metal, and are designed from a knowledge of the emission pattern of the target and the absorption characteristics of the filter material used

It very often happens that the characteristics of the beam as measured differ from those expected from the design, and it is common for the flattening filter to be altered experimentally (by removing metal at the appropriate places) in order to produce the desired degree of flatness This may be a difficult procedure as it involves removal of the flattener from the generator, a machining procedure to remove small amounts of metal from a complex shape, and subsequent replacement of the flattener in exactly the same position as before

Submitted for publication for 3 July 1972

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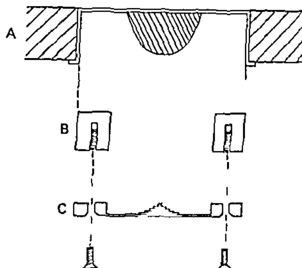


Fig 1 Mounting of auxiliary filter A) Turret with flattening filter B) Split ring C) Auxiliary filter

The linear accelerator in use at this hospital is the Mevatron 8 Model made by Applied Radiation Corporation This machine is capable of operation in 1 MeV steps from 3 MeV to 10 MeV Different flattening filters are available for each energy the flatteners are made up in the form of domes mounted in cups fitted into holes in a rotating turret, the appropriate flattener being automatically positioned by rotation of the turret on selecting the desired energy At present, we have used our machine only at 8 MeV

The manufacturers specification for this machine called for a flatness of ± 2 5 per cent over a 25 cm square field and preferably over a 30 cm square field In our case, the beam was found to be flat to ± 3 5 per cent over a 30 cm square field

Unfortunately most of this variation occurred in the middle portion of the field, resulting in the 95 per cent decrement line lying too close to the central axis of the beam and the 90 per cent decrement line being near the field edge giving rise to undesirable curvature of the isodose curves The obvious way to correct this defect would have been to add extra metal to the center of the beam flattener In our machine it was difficult to alter the flattener for the following reasons (1) The flattener is made of tungsten alloy small changes in dimensions are difficult to make especially since the flattener is mounted in a cup (2) It was deemed undesirable to remove the flattener from the machine to effect the necessary alterations as this was mechanically difficult it would have proved impossible to replace the flattener in exactly the same position as the cup was a loose fit in the turret hole and shims had been found necessary in order to position the flattener optimally

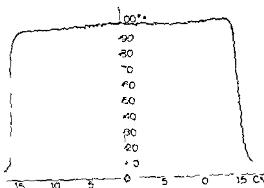


Fig 2 Beam profiles before (- -) and after (———) auxiliary filter has been fitted

For this reason, we made up an auxiliary flattening filter from thin brass sheets which were available in the department No attempt was made to provide a smooth correction, but the auxiliary flattener was made up in steps, by considering the intensity changes desirable over chosen areas of the beam, and the thickness of brass necessary to produce these changes In our machine, mounting of this auxiliary flattener was difficult The base of the main filter was inaccessible, and therefore the auxiliary filter had to be placed on the domed side There was less than 3 mm space available between the top of the dome and the surface of the turret, and little clearance was available between the turret surface and other components in the head of the machine, and it was therefore essential that the auxiliary flattener should be contained entirely within the main flattener cu[illegible] was no spac[illegible] environment [illegible] we therefore made a split ring, which could be fitted into the cup and expanded so as to grip the inside of the cup by driving a screw down a tapered tapped hole in the split The auxiliary filter was then mounted on a plate which could be screwed to the ring The arrangement is shown in Fig 1 Other mounting methods could doubtless be designed to suit other machines

In practice, thin sheets were cut to the appropriate size, and the filter made up by sticking these sheets together with cellotape The experimental filter could then be mounted easily, and tested by ionization chamber measurements in a water phantom When the flatness was deemed satisfactory, these sheets were replaced by a plate turned to the experimentally determined profile As it happened, the first attempt at correction was thought satisfactory, the technique proved very easy experimentally, and any changes necessary could be made and tested quickly The improvement produced by this technique is shown in Fig 2,

consisting of diagrams of the scans at 2 cm depth before and after fitting of the auxiliary flattener, and it can be seen the 95 per cent decrement line has been moved out very nearly to the edge of the beam The whole procedure took less than one afternoon's work and could easily be extended to any desired degree of accuracy The auxiliary flattener has been in position now for nearly two years, periodic inspection has revealed no slackening of the expander screw, and there appears to be no danger of the assembly falling out

Acknowledgement

Our thanks are due to Dr Henry P Plenk, Director of this Center, for his interest in this development and his patience in allowing us generous access to the accelerator

SUMMARY

A simple procedure is described whereby the flatness of the radiation from a linear accelerator may be improved without difficult operations on the flattening filter This is done by means of an auxiliary (or shimming filter) Alterations to the auxiliary filter are easily made and checked experimentally the technique could be applied to any other energy than the one investigated

ZUSAMMENFASSUNG

Ein einfaches Verfahren wird beschrieben mit dem die Feldhomogenitat der Strahlung von einem Linearaccelerator ohne schwierige Operationen am Ausgleichsfilter verbessert werden kann Das geschieht durch einen Hilfs oder Auffull Filter Anderungen am Hilfs filter sind einfach durchgefuhrt und experimentell uberpruft diese Technik konnte fur jede andere Energie verwendet werden nachdem die eine untersucht worden war

RÉSUMÉ

Les auteurs decrivent une technique simple permettant d ameliorer la planeite de la radiation d un accelerateur lineaire sans interventions difficiles sur le filtre de planeite Celui ci est realisé au moyen d un filtre additionnel (ou « shimming » filter) Il est facile de modifier le filtre additionnel et de controler experimentalement ces modifications cette technique pourrait etre appliquee a toute autre forme d energie que celle d un accelerateur lineaire

FROM RADIUMHEMMET AND THE DEPARTMENTS OF OTOLARYNGOLOGY, KAROLINSKA SJUKHUSET AND CLINICAL RADIATION PHYSICS, NATIONAL INSTITUTE OF RADIATION PROTECTION, 104 01 STOCKHOLM, SWEDEN

FRACTIONATION SCHEME WITH LOW INDIVIDUAL DOSES IN IRRADIATION OF CARCINOMA OF THE MOUTH

A BACKSTROM, P A JAKOBSSON, B LITTBRAND and J WERSÄLL

A previous report dealt with a theoretical possibility of increasing the efficiency of radiation therapy in respect of poorly oxygenated tumour cells by a modification of the fractionation scheme by low individual tumour doses (JAKOBSSON & LITTBRAND 1973) A scheme consisting of three treatments a day and a total dose of 8 400 rad (84 fractions/41 days) was suggested An investigation in which various fractionation schemes were used in the treatment of a patient with multiple basal cell carcinoma proved that this schedule could be applied without prejudice to the healing of normal tissue (JAKOBSSON & LITTBRAND) In the light of this experience it was deemed of interest to ascertain whether such a fractionation schedule is applicable in the treatment of carcinoma of the mouth

Material and Method The material comprised 17 patients The growths were staged according to the TNM classification (UICC, Geneva 1968), the primary sites in 5 patients was the lower gingiva, in 3 the upper gingiva, in 1 the floor of the mouth in 6 the front of the tongue and in 2 patients the root of the tongue

Submitted for publication 23 November 1972

consisting of diagrams of the scans at 2 cm depth before and after fitting of the auxiliary flattener, and it can be seen the 95 per cent decrement line has been moved out very nearly to the edge of the beam The whole procedure took less than one afternoon's work and could easily be extended to any desired degree of accuracy The auxiliary flattener has been in position now for nearly two years, periodic inspection has revealed no slackening of the expander screw, and there appears to be no danger of the assembly falling out

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Table 2

Nine patients (group B) initially considered as inoperable Mean tumor dose 8 400 rad given in split course therapy (4 200 rad/17 days in 2 sequences, cases 10 to 17) and without rest interval (84 fractions/44 days, case 9) Staging according to UICC (Geneva 1968)

Case	Sex	Age (yr)	Site of carcinoma	Stage	Post irradiation (mo)	Operation*	Clinical state
9	M	64	Lower gingiva	T4N1M0	27	+	Alive and well
10	F	62	Lower gingiva	T4N1M0	8	+	Alive and well
11	M	74	Lower gingiva	T4N1M0	19	+	Alive and well
12	F	84	Lower gingiva	T4N2M0	8	−	Alive with residual tumour
13	M	70	Upper gingiva	T4N0M0	12	−	Alive and well
14	M	73	Upper gingiva	T4N0M0	8	+	Alive and well
15	M	70	Tongue (oral)	T3N0M0	7	+	Alive and well
16	M	74	Tongue (root)	T3N1M0	17	−	Alive and well
17	M	67	Tongue (root)	T4N1M0	14	−	Alive with residual tumour

* The patients were operated on 1–2 months (in Case 9 13 months) after the irradiation

Results

Group A (Table 1) Incipient mucositis at a mean dose of 3 000 rad appeared in these patients receiving 4 200 rad and this became frank after 4 000 rad This condition had cleared up within an average time of 16 days (range 11 to 21 days aft[illegible]

[illegible] complications was recorded Two of the patients died from the carcinoma, one from distant metastases (Case 8) alone and the other from distant metastases and local recurrence (Case 5) Two died from intercurrent disease (Cases 1, 6) The other 4 patients were still alive after a mean observation period of 16 months (range 2 to 28 months) with no signs nor symptoms of malignancy (Cases 2, 3, 4 and 7)

Group B (Table 2) Incipient mucositis was evident after a mean of 2 900 rad in 8 patients treated with the split-course technique and became frank after 3 900 rad, during the second period of treatment mucositis appeared at 7 100 rad The confluent condition recurred in only 4 of the 8 patients, and then at the end of the irradiation Where the radiation therapy was carried out with no rest period (Case 9) mucositis appeared at 4 900 rad and the frank state failed to

Table 1

Eight patients (group A) initially regarded as operable Mean tumour dose 8 400 rad 42 fractions/17 days Staging according to UICC (Geneva 1968)

Case	Sex	Age (yr)	Site of carcinoma	Stage	Postoperative follow up (mo)	Clinical state
1	M	91	Lower gingiva	T3N0M0	11	Died from intercurrent disease
2	M	70	Upper gingiva	T4N0M0	2	Alive and well
3	M	61	Floor of the mouth	T4N0M0	28	Alive and well
4	F	81	Tongue (oral)	T2N0M0	8	Alive and well
5	M	36	Tongue (oral)	T3N0M0	12	Died from local recurrence
6	F	71	Tongue (oral)	T3N0M0	11	Died from intercurrent disease
7	M	65	Tongue (oral)	T3N0M0	26	Alive and well
8	F	84	Tongue (oral)	T3N1M0	16	Died from distant metastases

External irradiation was the rule with individual planning of the dose All the fields were irradiated at each treatment, the radiation source being a Siemens gammatron, a ^{60}Co unit, giving a dose rate of 40 to 60 rad/min at a distance of 60 cm The total tumour dose at each treatment was 100 rad and was repeated three times a day at intervals of four hours for five days a week

The series consisted of 2 groups Group A contained 8 patients in whom the tumour was considered to be operable and where the intended treatment was irradiation followed by operation The dose was 3 × 100 rad/14 days — a total of 4 200 rad over 3 weeks (Table 1) Group B comprised 9 patients who from the outset had been considered inoperable The dose was 28 × 3 × 100 rad — a total of 8 400 rad Except for one patient (Case 9) the therapy was given by the split-course technique, with a rest period at 4 200 rad of three weeks for 6 patients and five to seven weeks for 3 patients the longer interval was indicated by the age and general condition at the beginning of the treatment The exceptional Case 9 received 8 400 rad over 6 weeks with no rest period

A diagnosis of epidermoid carcinoma was always confirmed histologically before the treatment was begun Weak positive staining of the mucus strongly suggested mucoepidermoid carcinoma in Case 17



a b

Fig 2 Same case as in Fig 1 Appearance of the skin and tumour 12 months after irradiation

operation was performed one to two months after the irradiation The post-operative course was always uneventful and healing was normal The patients were discharged two to four weeks after the operation

The first patient of the series (Case 9) underwent operation 13 months after the irradiation when biopsy suggested recurrence A special microscopy of the surgical specimen however, disclosed no signs of residual malignancy

All 9 patients in Group B were alive after a mean observation period of 13 months The treatment in 2 of the 9 patients was regarded as palliative from the outset in one of these (Case 17) the tumour involved the whole of the anterior floor of the mouth with bone destruction and penetration of the skin The radiation therapy resulted in marked regression of the condition which then remained stationary for 6 months when it increased again The second patient (Case 17) had an inoperable tumour involving the whole of the dorsum and root of the tongue with extensive ulceration, and giving rise to cervical metastases The irradiation produced marked regression of the main mass and the ulceration cleared up although deeper in the tongue palpable and doubtless malignant tissue remained The tumour then remained stationary for a year after the irradiation was discontinued

Discussion

The preliminary results indicated that a total dose of 8 400 rad delivered by a fractionation scheme with three treatments daily and 100 rad per treatment



Fig 1 Case 9 Male aged 64 with carcinoma of the lower gingiva invading the floor of the mouth and causing extensive destruction of the mandible (T4N1M0) Total tumor dose 8 400 rad State of the skin and tumour before irradiation

develop until the end of the irradiation This patient had carcinoma of the lower gingiva (Fig 1) with extensive destruction of the mandible (T4N1M0) The dose totalled 8 400 rad with no rest interval The skin of this patient before and 12 months after irradiation is depicted in Figs 1 a, 2 a The normal mucosa at the same site after healing of the irradiated region 12 months after the radiation therapy appears in Fig 2 b It is evident from the illustrations that the normal tissue healed without sequelae in spite of the fact that the high total dose was administered in a short period of 6 weeks

The patients in this group were considered to be inoperable on account of the extent of the tumour In 5 of the 9 patients in whom irradiation rendered operation possible and malignant remnants probable, operation was carried out (Cases 9, 10, 11, 14 and 15) This in Case 9 consisted of en bloc resection with the mandible, part of the tongue and base of the mouth on the left side and with partial cervical dissection In Case 10 en bloc removal of the left side of the mandible with part of the bucca and neck dissection was performed, Case 11 had en bloc resection of the mandible, cheek and base of the mouth on the left side with cervical dissection and reconstruction with forehead flap and acromipectoral flap In Case 14 local excision of the alveolar process and the right side of the hard palate was performed while Case 15 was subjected to tongue resection and cervical dissection on the same side Except in Case 9 the

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APPROACH TO A THEORETIC MODEL FOR THE PROBABILITY OF RECURRENCES IN RADIATION THERAPY

E SPRING and K PAASIKALLIO

Attention has frequently been paid in the application of radiation therapy to tumours to a relatively high and uniformly distributed dose being obtained Investigations of carcinoma of the lung made by HOLSTI et coll (1971) have indicated that the irradiation should cause cell death corresponding to a cell survival fraction S below a certain value $S \approx 10$ if the tumour is to be removed For the attainment of a high probability of successful treatment, the cell survival fraction should be under an even lower value, that is $S \approx 0.3 \times 10^{8}$ The survival values indicated were calculated by means of the so-called single hit multi target model with parameter values of $D_0 = 160$ rad and $m = 2$

Investigations of carcinoma of the breast made by COHEN et coll (1971) have demonstrated that any schedule of treatment that yields an estimated survival value below $S \approx 10$ almost guarantees success The survival values were calculated by application of the cell population kinetic model and a computer

Submitted for publication 6 November 1972

was not during the observation period followed by impaired local healing. In general, however, mucositis appeared to such an extent that a rest interval of about three weeks in the middle of the treatment was considered desirable. In the split-course therapy the second sequence of the treatment appeared always preferable to the first from the viewpoint of the patient, although the total doses were the same in both. No late injury was recorded but it should be borne in mind that the mean observation period averaged only 13 months, and never exceeded 27 months. No increase in complications nor delayed healing was apparent when an operation was performed (BÄCKSTRÖM et coll. 1972). The preliminary results obtained with the fractionation scheme described confirm the conclusions drawn from the results of the irradation of basal cell carcinoma wth the same scheme (JAKOBSSON & LITTBRAND).

Notwithstanding the short observation time the absence of serious sequelae would seem to justify this method being applied to a larger material

SUMMARY

A modified fractionation scheme with low individual doses has been tested in a preliminary investigation of 17 patients with carcinoma of the mouth Late injury was not apparent The observation time averaged thirteen months, and never exceeded twenty-seven months Operation, when performed, produced no untoward complications nor delayed healing

ZUSAMMENFASSUNG

Ein modifiziertes Fraktionierungsschema mit niedrigen Einzeldosen wurde in einer Prelinaruntersuchung bei 17 Patienten mit einem Mundkarzinom vorgenommen Es traten keine Spatschaden auf Die Beobachtungszeit betrug durchschnittlich 13 Monate und uberschritt in keinem Fall 27 Monate Sofern eine Operation vorgenommen wurde, traten keine unerwarteten Komplikationen oder eine verzogerte Heilung auf

RÉSUMÉ

Les auteurs ont utilisé un schéma modifié de fractionnement avec de petites doses partielles dans une expérimentation faite sur 17 malades atteints de cancer de la bouche Il n'y a pas eu de lésion tardive Le temps d'observation a été en moyenne de 13 mois et n'a jamais dépassé 27 mois L'opération, quand elle a été faite, n'a pas donné de complications et n'a pas retardé la guérison

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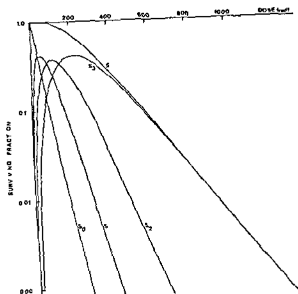


Fig 2 Semilog plot of a single hit multi target curve S (4 targets D_0 160 rad) Curve $S = 1-(1-e^{-D/D_0})^m$ is the sum of the fractions of the population that survive with 0 to 3 targets hit (ELKIND & SINCLAIR 1965)

$$S = 1 - (1 - \exp(-D/D_0))^m \qquad (1)$$

in which D_0 is the 37 per cent dose, and m is the extrapolation number or the number of sensitive targets in the cell, D_0 is the absorbed dose required to reduce the surviving population to 37 per cent of the original number on the straight part of the survival curve The word target should not be understood in the strict sense of an 'anatomic target', but in a broader one of sublethal injury, the accumulation of which leads to death of the cell

It would perhaps be more realistic to use a combination of single hit and multi-hit curves or for the sake of simplicity in calculations, to employ a combination of single hit, single target (S_1) and single hit, multi target (S_m) cell survival models such as

$$S = S_1 S_m = \exp(-D/D_1) \times (1 - (1 - \exp(-D/D_m))^m) \qquad (2)$$

with the slope at large doses $\frac{1}{D_0} = \frac{1}{D_1} + \frac{1}{D_m}$, and extrapolation number m

(BARENDSEN 1967) for description of the survival of the irradiated cells Nevertheless, in the present investigation it would have been impractical to consider that the phenomenon connected with the S_1 component would have influenced the results derived, it is assumed that those cells have one target, which needs a single hit for the cell to be killed

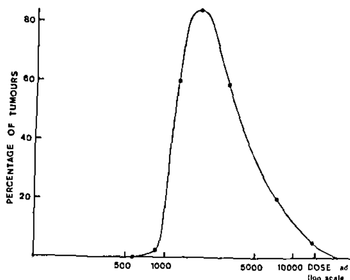


Fig 1 Percentage of rats bearing renal tumours as a function of dose (MALDAGUE 1969)

programme described by COHEN & SCOTT (1968) with the insertion of parameters (mean cellular lethal doses, extrapolation numbers and generation times) appropriate to normal human skin and mammary carcinoma

The authors have been endeavouring to find a theoretic estimate for this phenomenon the appearance of recurrences despite irradiation It is assumed that their origin might be too high a proportion of radiation-damaged cells in the tumour or radiation induced carcinoma in the normal tissue surrounding it The *main purpose of the present investigation was to try to develop a mathematical* model for the frequency of recurrences as a function of the cell survival values induced by the radiation dose The final aim was the discovery of a means of calculating the risk of recurrence in the irradiated region borne by a patient subsequent to treatment

The calculations have been based upon the results obtained in the experiments by MALDAGUE (1969) who in mice and rats observed the inducement of carcinoma in a kidney that had been irradiated (Fig 1) During the preparation of this report, another investigation by SPRING et coll (1972), on carcinoma of the larynx, indicated that the number of recurrences increased if the cell survival fraction fell below $\approx 0\,3 \times 10^{-9}$ This also emphasized the necessity of theoretic analyses of experimental results concerned with recurrences in radiation therapy

The assumption has been made that the survival fraction S of the cells after irradiation by ionizing radiation, may be described by *the familiar single-hit,* multi-target model,

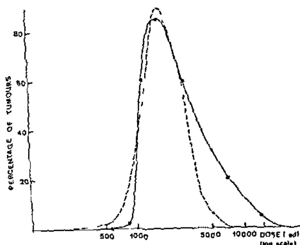


Fig 4 Best fit of equation (3 a) obtained for the induced renal tumours (Fig 1) as a function of dose

Fig 3 represents almost the same situation with the distribution of the irradiated cells as a function of hit targets after different doses of radiation. The left bar of each diagram indicates the survival fraction, and the others represent the hit distribution of the surviving cells (as a percentage of the surviving cells, which have 0, 1, 2, $m-1$ targets hit).

The model proposed Fig 1 illustrates the percentage of rats with kidney tumours as a function of the dose (MALDAGUE 1969) The doses from 0 to 570

... radiation lesions were always produced by these doses The maximal carcinogenic effect was reached at 1 710 rad, which MALDAGUE states is the optimal dose for radiation induced renal carcinoma Excessive doses from 7 000 to 14 000 ... seldom and ... 14 250 rad ... to kidney, and a solid tumour proved to be a sarcoma of the perirenal tissues Since the tumour appeared in perirenal tissues, it is possible that the dose absorbed in that volume had been less than that of the irradiated kidney (14 250 rad) For this reason, the end section of the curve (Fig 1) should perhaps be a little lower, after such doses the frequency of the induced carcinoma of the kidney approaches zero

It ...

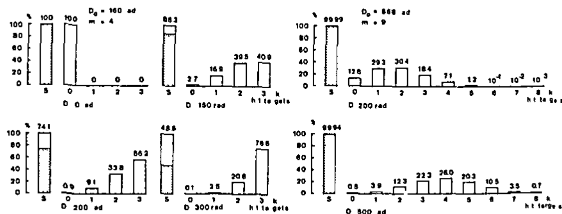


Fig 3 Distribution of the irradiated cells as a function of hit targets after different doses of radiation The calculations were made with equation (3 a) and the single hit multi target model with a) $D_0 = 160$ rad and $m = 4$ and b) $D_0 = 868$ rad and $m = 9$ The left bar of each diagram indicates the surviving fraction S (%) of the irradiated cell population

Equation (1) may also be derived (Elkind & Sinclair 1965) by summation of the fractions of cells surviving with 0, 1, , m-1 targets inactivated, since according to the model a cell is not killed until all m targets are hit The probability of a target being hit after a dose D is $P = 1 - \exp(-D/D_0)$, and the fraction of cells with k hit targets and $m-k$ intact targets is (Wambersie et coll 1970)

$$S_k = \frac{m!}{k!\,(m-k)!}\,(1 - \exp(-D/D_0))^k(\exp(-D/D_0))^{m-k} \qquad (3a)$$

Thus the total survival probability given by equation (1) is also obtainable from

$$S = \sum_{x=0}^{m-1} \binom{m}{x} (1 - \exp(-D/D_0))^x(\exp(-D/D_0))^{m-x} \qquad (3b)$$

The dose dependences of S_k ($m = 4$, $D_0 = 160$ rad) is depicted in Fig 2 In the shoulder region of S, that is $0 < D \leqslant 3D_0$, all of these $m - 1$ components make significant contributions to S With the exception of S_0, the other components $S_1, S_2, \ldots S_{m-1}$ represent the dose dependence of cells surviving with different degrees of sublethal damage, since 1 to $m - 1$ targets have been hit Many cells survive, with varying amounts of sublethal damage in the 'shoulder' region Where S_{m-1} approaches S, the likelihood of cells surviving with less than a saturation amount of damage ($m - 1$ hit targets) becomes quite small As a consequence all the surviving cells essentially require one additional hit target to be inactivated, thus S ($\approx S_{m-1}$) becomes exponential, and has a constant slope of $1/D_0$ (Elkind & Sinclair 1965)

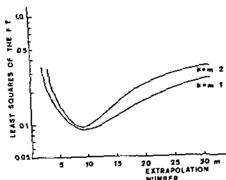


Fig 5 Least squares of the fitting of equation (3 a) to the curve of Fig 1 as a function of the extrapolation number

and 0 75, these are similar to those given by the single hit, multi target model with parameter values of $m = 9$ and $D_0 = 868$ rad ($S = 0\,9$ and $0\,7$ respectively) on conversion of the exposure in roentgen to the absorbed dose in rad The ...

... squares of the fit in Fig 5 The minimum of this latter curve *is not unduly* sharp and thus no exact fitting is achieved but, as was mentioned, the last part of the curve may be slightly unreliable for statistical reasons (twenty rats with 1 tumour in the perirenal tissues at 14 250 rad)

Since the minimum of the least squares of the fit displays a gentle slope, the value for m may equally well be chosen as value from 7 to 12, although the best fit was at $m = 9$

Critical dose As with recurrences the most critical dose is attained when the absolute number of cells with maximal sublethal damage reaches a maximum value, equation (4) is reducible to a somewhat simpler expression by insertion of the value $l = n - 1$

$$p = g\ n\ (1 - \exp(-D/D_\circ))^{n-1}(\exp(-D/D_\circ)) \qquad (5)$$

The maximal tumour frequency appears when this equation has its maximal value The most critical tumour inducing dose is thus obtainable by

$$\frac{d}{dD}(p) = \frac{g\ n}{D_\circ}\exp(-D/D_\circ)(1 - \exp(-D/D_\circ))^{n-2}(n\ \exp(-D/D_\circ) - 1)$$

Table

Percentage weight reduction in the irradiated compared with the non irradiated kidney 2, 3 4 and 5 months after the radiation (FEINE 1963)

Dose R	2 months	3 months	4 months	5 months
500	5	5	5	5
1 000	5	5	5	5
1 500	5	10	8	26
2 000	25	48	45	55

attempt was made to fit the curve in Fig 1 by application of the function given by equation (3 a) The best fit obtained was that of equation (3 a) multiplied by a normalizing factor g (Fig 4)

$$p = g \; \frac{n!}{l!\,(n-l)!} \, (1 - \exp(-D/D_0))^l (\exp(-D/D_0))^{n-l} \qquad (4)$$

The parameters were given values that ranged as follows g from 1 9 to 4 0, D_0 from 150 to 1 500 rad, n from 2 to 30 and l from 1 to 29 The values that gave the best fit were $g \approx 2\ 15$, $n = 9$, $l = 8$ and $D_0 = 868$ rad This indicates that the cell of a rat kidney has 9 'targets', furthermore the probability that an induced tumour will appear is proportional to the amount of cells which have survived with a saturation amount of damage, i e 8 targets have been hit Thus the most critical point for the inducement of tumours was reached when the absolute number of cells with eight of their nine targets hit were at maximum value Nevertheless, it is extremely probable that only a small percentage of the cells surviving with maximal sublethal damages prove to be those that give rise to tumours The parameter values $n = 9$ and $D_0 = 868$ rad provide an indication of very radiation resistant tissue, this might even be the case as most kidney cells are nonproliferating

FEINE (1963) has also investigated the effects of radiation on rat kidneys with single doses of 500, 1 000, 1 500 and 2 000 R The Table illustrates the weight reduction in the irradiated kidney (only half of the kidney was irradiated the other half being screened), compared with the non-irradiated kidney The reduction in weight became apparent after 3 months when the dose was 1 500 R, and after 2 months when it was 2 000 R It is assumed that the reduction in weight was proportional to the survival fraction of the irradiated kidney cells, that is, if the weight reduction is 10 per cent the corresponding survival value will be 0 9 The reductions in weight when they first appear with doses of 1 500 and 2 000 R, are 10 and 25 per cent, consequently the survival values are 0 9

ZUSAMMENFASSUNG

Das Phanomen des Rezidivs bei der Strahlentherapie wurde an theoretischen Modellen untersucht und ein Ansatz fur eine Erklarung gemacht, die auf dem Eintreff multipel Treff bereich Prinzip beruht Es scheint dass die Wahrscheinlichkeit fur ein Rezidiv am grossten ist wenn die absolute Zahl von Zellen mit einem intakten Treffbereich ihren hochsten Wert erreicht Es wird eine Formel fur eine Rezidiv Risiko-Dosis vorgelegt

RÉSUMÉ

Les auteurs ont etudie le phenomène de recidive en traitement par les radiations grâce a des modeles theoriques et ont etudie une explication basee sur le principe de coup unique sur cibles multiples Il semble que la probabilite des recidives est plus grande quand le nombre absolu de cellules avec une cible intacte atteint sa valeur maximale Les auteurs proposent une formule pour calculer la dose donnant un risque de recidive

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SPRING E SALMO M and RISSANEN P M The effect of ... [illegible]

W[illegible]

The critical value of D can be derived from the following equation

$$\frac{d}{dD}(p) = 0,$$

which gives

$$D = D_0 \ln n \qquad (6)$$

On application of the parameters mentioned D becomes $D = 868$ (rad) $ln\, 9 =$ 1 907 rad, and the difference from the right value (Fig 4) is approximately $D = |1\,907 - 1\,710| = 197$ rad and $|\Delta D/D| = |197/1\,710| \approx 12\,\%$ MALDAGUE gives no values for experimental errors, but only one graphical expression It seems that the D value obtained probably falls within the limits of experimental error

Discussion

The formulas proposed and the calculations made must be taken as an attempt to calculate the probability of recurrences in radiation therapy The investigation was based upon theoretic models of the influence of ionizing radiation upon tissue applied with a view to finding a theoretic means of describing the recurrence phenomenon The parameter values presented are valid for rat kidneys, and consequently cannot be used direct with human tissues Another limitation was that the rats had been irradiated with single doses and not with some form of fractionated doses, as normally happens with human subjects However this does not affect the observation that the induced tumours appeared most easily when the absolute number of cells with maximal sublethal damage reached its maximum value

In comparison with irradiated human tissues, the dangerous cells that may give rise to new tumours or recurrence are those tumour cells that survive treatment and the cells of the surrounding tissues that have suffered maximal sublethal damage

Acknowledgements

The authors wish to thank the Academy of Finland for financial support

SUMMARY

The recurrence phenomenon in radiation therapy has been investigated by theoretic models, and approach made to an explanation based upon the single hit multi target principle It seems that the probability of recurrence is highest when the absolute number of cells with one intact target attains its maximum value A formula is proposed for a recurrence 'risk dose

Method The arrangement of equipment and patient for the investigation is shown in Fig 1 The patient lies on his back with the gamma camera placed below a low attenuation bed A planar source containing 10 mCi $^{99}Tc^{m}$ pertechnetate is placed at a distance of 40 cm above the bed The collimator used on the camera ensures that only photons passing approximately perpendicularly through the chest are detected Different areas of the chest will absorb different amounts of radiation depending on the mean density and thickness of the section of tissue below the area as in a conventional roentgenogram The patient breathes in and holds his breath for 10 s while an image is obtained and counts are accumulated on the 64×64 channel memory unit in the positive mode After allowing the patient to breathe normally for a period he is instructed to breathe out and hold the expiration position for a further 10 s while a second image is obtained Counts are now accumulated on the memory unit in the negative mode The final image displayed, which is a subtraction of the expiration distribution from that obtained during inspiration is a measure of the regional ventilation of the lung The above procedure is repeated a number of times in order to obtain sufficient counts for a statistically smooth image

Experiments with the source and tissue equivalent material showed that the 140 keV emission of $^{99}Tc^{m}$ was about the optimal energy At lower energies the number of photons passing through the chest is severely reduced and at high energies the detection efficiency of the sodium iodide crystal of the camera decreases rapidly The results are given in Table 1

Theory The theory of the technique can be simply understood by considering the lung as cylinder within which a piston moves (Fig 2) On inspiration the diaphragm is displaced downwards and in a ventilating lung the tissue becomes filled with air and expands into the increased volume Hence the mean density of tissue across the lung is decreased If the lung is not ventilating the lung tissue does not expand and the density across the lung is unaltered The density changes in a particular area of the lung are, therefore, related to the ventilation of that area The density changes produce a difference in the counts accumulated on inspiration from those on expiration Since this difference is recorded in the final subtraction image we have a measure of the distribution of density changes and hence of regional ventilation

More rigorously we can consider the count rate (N_A) of area A of the lung to be given by

$$N_A = N_0 \exp\left[\frac{\mu}{\rho}(L\rho)\right] \quad (1)$$

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REGIONAL VENTILATION ASSESSMENT BY TRANSMISSION SCINTIGRAPHY

A preliminary report

J S FLEMING, B A GODDARD and D L RAYNER

The ability to obtain information on pulmonary ventilation has proved to be of clinical value, especially when used in conjunction with the perfusion lung scan in the differential diagnosis of pulmonary embolism

So far, three main methods have been used in ventilation assessment The first, and most popular technique, is to use a radioactive gas such as ^{133}Xe Both arrays of counters (WEST 1966) and gamma cameras (MEDINA et coll 1969) have been used as detectors An alternative method (PIRCHER 1969) is to use a radioactive aerosol which labels ventilating areas of the lung over a long enough period for the area to be scanned More recently, a third technique involving the measurement of pulmonary density changes by differential transmission has been introduced by POTCHEN et coll (1970)

Both of the first two techniques mentioned involve periods of about 10 minutes breathing with a mask on The third offers a simple and rapid technique of obtaining information on ventilation This report describes an adaption of POTCHEN's technique for use with a gamma camera and memory unit with subtraction facilities, and compares it with the ^{133}Xe gas inhalation method

Submitted for publication 28 July 1972

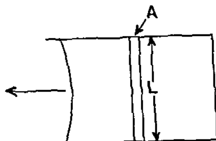


Fig 2 Diagram of a lung represented as a cylinder with a moveable piston

The mean density of the tissue and bone through the thorax is approximately 1 04 This means that the volume of air below area A on inspiration is

$$LA(1\ 04 - \varrho_I)/1\ 04$$

Similarly on expiration it is

$$LA(1\ 04 - \varrho_E)/1\ 04$$

Hence the volume of air moved on respiration, i e the ventilation of the area is

$$LA(\varrho_E - \varrho_I)/1\ 04$$

Table 1

Comparison of statistical significance of counts obtained on transmission with isotopes of different energy

	^{133}Xe	^{99}Tcm	^{113m}In
10 s count difference for density change from 0 7 to 0 8 in units of standard deviation	22 9	33 7	15 7

Table 2

Comparison of results obtained from the transmission technique and the ^{133}Xe inhalation method

	Xe gas wash in defect	Xe gas wash out defect	Defect on both phases	No defect in Xe method
Defect observed on transmission	19	7	9	1
Defect not observed on transmission	0	3	0	11



Fig 1 Arrangement of patient and equipment

where N_0 is the count rate from the corresponding area of the source and $\frac{\mu}{\varrho}$ is the mass attenuation coefficient

For a mixture of air and tissue

$$\frac{\mu}{\varrho} = \frac{\mu_a}{\varrho_a} W_a + \frac{\mu_T}{\varrho_T} W_T$$

where W_a and W_T are the fractions by weight of air and tissue present (EVANS 1955)

Since W_a is much smaller than W_T it follows that

$$\frac{\mu}{\varrho} \approx \frac{\mu_T}{\varrho_T}$$

Assuming L is constant, the difference in count rate in area A between inspiration and expiration is

$$N_0\left(\left(\exp\left[\frac{\mu_T}{\varrho_T}(L\varrho_I)\right] - \exp\left[\frac{\mu_T}{\varrho_T}(L\varrho_E)\right]\right)\right)$$

This difference in count rate is measured on the subtraction image and is clearly related to the density change





Fig 5 Comparison of single breath xenon examination (left) with transmission subtraction (right) for a patient with an apex defect

gave values of lung volume within 5 per cent of those obtained using a standard spirometer method

Results

The test was performed on 50 patients, all of whom had also been examined with the ^{133}Xe gas inhalation ventilation method The latter gives information on areas of the lung which are badly ventilating during the wash-in phase of the gas and distinguishes them from areas such as those in emphysematous conditions where the wash-out phase is prolonged The transmission subtraction image,





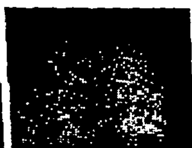


Fig 6 Comparison of single breath xenon examination (left) with 5—6 min wash out (middle) and with transmission subtraction (right) for a patient with a wash-out defect

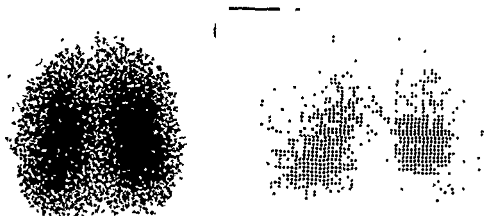


Fig 3 Comparison of single breath xenon examination (left) with transmission subtraction image (right) for a normal

With the present technique we are not measuring actual density changes However, using computer facilities to apply eq (1) to each area of the lung, regional density could be measured for both the inspiration and expiration pictures The value of the mass attenuation coefficient for tissue is measured empirically using tissue equivalent material Regional lung volume could be calculated for both states provided that the distribution of chest thickness is known and, therefore, the actual volume of air moved on respiration at different parts of the lung may be obtained Initial investigations using this method on three normal volunteers



Fig 4 Comparison of single breath xenon examination (left) with transmission subtraction (right) for a patient with a base defect

Comparison of the transmission subtraction images with the single breath examinations in normals reveals that the pattern is similar (Fig 3) However, a detailed quantitative analysis of the two distributions of counts shows that they are not exactly the same This is due to the different amounts of tissue absorption in the two tests Also the transmission image is affected by the variation in pulmonary tissue expansion over the area of the lung, which depends on whether the breathing is primarily costal or abdominal

The areas of largest expansion on inspiration are at the base of the lung and the largest density changes are observed here If the diaphragm movement is larger than the resolution of the camera, i e about 4 to 5 cm, a bright band is observed at the base of the image corresponding to the increase in lung size on inspiration In three patients of the series this bright band was observed, but in none of these cases was a base defect obscured Fig 4 gives an example of a patient with a defect at the left base which is observed by the subtraction technique The smaller number of counts in the apex region might have obscured defects here, but this was very rarely the case Fig 5 shows the comparison of the single breath xenon ventilation and transmission images for a patient with a defect in the right apex which was detected by both techniques

Although specific wash-out information is not available with the transmission technique the subtraction image does contain wash-out information Fig 6 shows the subtraction image for a patient with emphysema and reveals defective ventilation in the left lung which was verified with the ^{133}Xe gas inhalation method in the wash-out phase

In one patient with bronchial carcinoma, a large volume of trapped air, which was virtually stationary on respiration was observed In Fig 7 the left base is seen to contain air from the transmission inspiration image However the subtraction image shows this to be a region of poor ventilation which was confirmed in the ^{133}Xe examination

The major advantage of the technique over the xenon method is that it is much quicker and easier to perform and could be used as a screening technique Also the radiation dose, approximately 1 mrad to the lungs is very much less than that for the xenon ventilation examinations, which is about 500 mrad There are no blood solubility errors as in the xenon technique and sophistication of the method using computer manipulation of the data could give information on regional lung volume which is not always obtained with the xenon method

The primary disadvantage is that less specific information is available with the technique in its present form Data handling equipment is also required and the test cannot be performed on patients who cannot hold their breath The small number of counts obtained makes interpretation of the images more difficult Both techniques suffer the limitations of resolution of the gamma camera

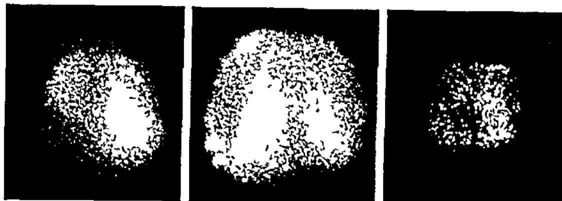


Fig 7 Comparison of 1—2 rebreathe (left) with transmission (middle) and with transmission subtraction (right) techniques for a patient with a volume of trapped air

being a difference between inspiration and expiration density, gives both wash in and wash-out information but does not distinguish between them Table 2 gives a summary of the comparison of the two techniques In general there was a very good agreement between the two methods, the main discrepancy being the inability of the transmission technique to detect small wash-out defects in three cases However, the xenon images gave the information in a clearer form, partly due to the low number of counts obtained from the subtraction image

Discussion

There are two major limitations to the technique at present (1) it does not give a direct quantitative measure of lung volume changes as considered in the theory, and (2) the number of counts obtained in the subtraction image is generally too small for a statistically smooth image unless several inspiration and expiration images are used

There is also an error due to the expansion of the non pulmonary tissue on inspiration During inspiration the diaphragm moves downwards and the lung tissue expands into the increased volume At the same time the thorax may be enlarged from back to front and from side to side resulting in a small decrease in the thickness of the chest wall This will tend to give a falsely high value to the counts on inspiration as compared to the expiration image The extent of this effect will depend on whether the respiration was costal, abdominal or both These uncertainties, together with the resolution of the gamma camera, limit the technique to the detection of gross ventilation defects of the order of several centimetres

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EFFECT OF RADIOPROTECTIVE DRUGS ON THE THERAPEUTIC RATIO FOR A MOUSE TUMOR SYSTEM

R O LOWY and D G BAKER

Means have been sought to increase either the radiation sensitivity of tumor cells or the radiation tolerance of normal tissues in attempts to improve long term therapeutic control of malignant tumors The sulfhydryl family of radioprotective agents has been reported to preferentially protect fully oxygenated normal tissues as compared with hypoxic neoplastic cells (COHEN & COHEN 1959) This increases the resistance of normal tissues to irradiation while not appreciably increasing the tumor's radiation resistance Such a differential would allow the delivery of a higher dose of radiation to the tumor, thereby improving the therapeutic ratio the relationship of normal tissue tolerance to tumor lethal dose Earlier investigations have shown only a slight differential protection of normal tissues, which was probably related to the weak protective effect of the compounds investigated (SCHWARTZ et coll 1964) Statistical improvement in the differential protection has however recently been found with new protective agents (YUHAS & STORER 1969)

Submitted for publication 10 October 1972

In conclusion, it is not to be recommended that the test in its present form could completely replace the ^{133}Xe gas inhalation technique as it does not give such explicit information. However we have demonstrated the validity of the test and the advantages described above could lead to its usefulness in certain situations. Moreover, it is considered that the computer processing of the data could make this an even more useful clinical test.

SUMMARY

A technique for the assessment of regional ventilation is described. A gamma camera placed below the patient detects radiation transmitted through the chest from a planar source situated above. Subtraction of the counts obtained after full expiration from those in the same period of full inspiration gives a measure of regional ventilation. Comparison with the ^{133}Xe gas inhalation method shows that there is good correlation between the two techniques.

ZUSAMMENFASSUNG

Eine Technik zur Bestimmung der regionalen Ventilation wird beschrieben. Eine unter dem Patienten aufgestellte Gammakamera misst die durch den Brustkorb durchgelassene Strahlung von einer gleichmässigen Quelle, die oberhalb angebracht ist. Die Subtraktion der Impulse die nach vollständiger Expiration erhalten werden, von denen in der gleichen Periode einer vollständigen Inspiration bildet ein Mass der regionalen Ventilation. Im Vergleich mit der ^{133}Xe Gas Inhalations Methode zeigt, dass eine gute Korrelation zwischen diesen beiden Techniken vorliegt.

RÉSUMÉ

Description d'une technique de détermination de la ventilation régionale. Une gamma caméra placée sous le patient détecte le rayonnement transmis à travers la poitrine provenant d'une source plane située au dessus. La différence entre les comptages obtenus après expiration complète et après inspiration complète donne la mesure de la ventilation régionale. La comparaison avec la méthode d'inhalation de gaz ^{133}Xe montre qu'il y a une bonne corrélation entre ces deux techniques.

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R O LOWY and D G BAKER

Means have been sought to increase either the radiation sensitivity of tumor cells or the radiation tolerance of normal tissues in attempts to improve long term therapeutic control of malignant tumors The sulfhydryl family of radioprotective agents has been reported to preferentially protect fully oxygenated normal tissues as compared with hypoxic neoplastic cells (COHEN & COHEN 1959) This increases the resistance of normal tissues to irradiation while not appreciably increasing the tumor's radiation resistance Such a differential would allow the delivery of a higher dose of radiation to the tumor, thereby improving the therapeutic ratio the relationship of normal tissue tolerance to tumor lethal dose Earlier investigations have shown only a slight differential protection of normal tissues which was probably related to the weak protective effect of the compounds investigated (SCHWARTZ et coll 1964) Statistical improvement in the differential protection has however recently been found with new protective agents (YUHAS & STORER 1969)

Submitted for publication 10 October 1972

This investigation has been designed to find out the ability of cysteamine (2 mercaptoethylamine or MEA) and WR-2721 [S-2- (3-aminopropylamino) ethylphosphorothiotic acid hydrate] to modify the therapeutic ratio This paper reports the success of combined drug and radiation therapy in achieving long term tumor control in a mouse tumor system

Materials and Methods

Female C3H/HeJ mice, 10 weeks of age and 20 grams in weight at the time of irradiation, were used All animals were offered standard laboratory chow and water ad libitum

The tumor used was the KHT sarcoma, which arose spontaneously at the base of the ear in a C3H-Km mouse (KALLMAN et coll 1967) The tumor line has been maintained for three years in this laboratory by serial passage of 1 mm^3 tumor fragments, implanted subcutaneously into the axilla of normal 10 week-old recipients The transplanted tumor line is stable with no changes in cell type or virulence The tumor has a consistent anoxic component between 14 per cent and 18 per cent (VAN PUTTEN & KALLMAN 1968) Subcutaneous inoculations of *100 000 KHT tumor cells will produce a palpable 1 mm nodule in 7 to 8 days*, and a 10 mm diameter mass in 12 to 14 days If untreated, the tumor remains localized, growing to a size of 25 to 35 mm diameter in 21 days, when most animals die without metastases

Throughout this investigation, the tumor was transplanted by injecting tumor cell suspension subcutaneously in the skin of the lower leg Ten millimeter tumors are excised from donor animals, freed of non-tumor debris, minced with fine *scissors, extruded through gauze mesh, and diluted with ice-cold* Hank s solution Under phase microscopy, counts of viable cells were made by using trypan blue as a dilutant The suspension was then adjusted to 2×10^6 viable cells per ml, and 0 05 ml were injected subcutaneously with a microsyringe under the skin of the right lateral thigh

Animals selected for irradiation had subcutaneous tumors *measuring 10 mm* in diameter The hair of the entire leg was removed with a depilatory agent They were anesthetized with Diabutal injected intraperitoneally Four animals were irradiated simultaneously in a special holder with lead shielding, so as to expose the entire lower limb, allowing 1 cm margin superior to the edge of the tumor Irradiation data 250 kVp (1 25 mm Cu HVL), dose rate *200 rad per* minute at a SSD of 35 cm The first irradiation was delivered within one hour of the hair removal *All animals treated received two equal fractions* with a 48-hour interval, the approximate doubling time of the tumor

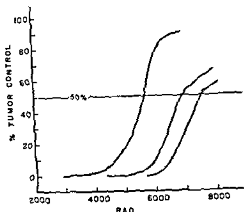


Fig 1 Per cent of animals whose tumors were controlled The curves from left to right represent irradiated unprotected animals and animals irradiated and protected with WR 2721 and cysteamine respectively

An amount of 175 mg/kg of MEA was injected intraperitoneally in 0 1 ml of sterile, distilled water 15 minutes prior to each treatment WR 2721 was also given intraperitoneally at a dose of 400 mg/kg 15 minutes prior to exposure To avoid acute toxicity with WR 2721, the anesthetic dose was reduced by 50 per cent

Long term tumor control The radiation dose (100 days without recurrence) was estimated by irradiating the tumors in animals to doses ranging from 2 500 rad to 7 000 rad in two fractions Groups of 10 to 20 animals were used at each of the dose levels Tumors in groups of animals were irradiated with simultaneous administration of cysteamine or WR-2721 Tumor size and normal tissue reactions were recorded All animals were kept until death and autopsied

Acute injury to the skin Assessment in non tumor bearing animals was evaluated as an index of the response of a normal tissue Three separate conditions were studied animals irradiated without drug, those irradiated with cysteamine, and those irradiated with WR-2721 Groups consisting of 15 animals were irradiated with a similar fractionation scheme at each dose level, which ranged from 2 000 rad to 9 000 rad in two fractions Skin reactions were graded and recorded daily by a single observer without the knowledge of the treatment received, until the entire acute phase was completed at 21 to 35 days (VAN DEN BRENK 1966) The skin reactions were graded as follows grade 1 no reaction, grade 2 early mild erythema, grade 3 strong erythema, grade 4 dry desquamation, grade 5 moist desquamation, grade 6 ulceration and necrosis

Table

Number of tumor-bearing animals irradiated without drugs (nonprotected), after Cysteamine (MEA protected), and after WR-2721 (WR-2721 protected). The time until recurrence or death is an average value with standard deviation. Where numbers were small a range of time rather than the standard deviation is shown

Radiation dose (rad)	Controlled*		Time in days	
	No	%	Until recurrence	Recurrence until death
Nonprotected				
2500	0/8	0.0	12 ± 1.8	21 ± 2.1
3000	0/8	0.0	16 ± 0.9	25 ± 1.3
3500	0/8	0.0	20 ± 1.2	25 ± 1.6
4000	0/10	0.0	20 ± 1.6	31 ± 1.0
4500	1/12	8.3	20 ± 1.5	29 ± 1.4
5000	2/16	12.5	37 ± 2.4	29 ± 3.[illegible]
5500	7/15	46.7	42 ± 4.4	30 ± 2.[illegible]
6000	10/12	83.0		
7000	8/9	89.0		
MEA protected				
4500	0/11	0.0	15 ± 1.2	31 ± 2.1
5000	0/14	0.0	18 ± 1.0	27 ± 2.7
6000	0/10	0.0	20 ± 0.8	30 ± 4.1
7000	4/11	36.0	24 ± 2.6	28 ± 3.2
8000	4/7	57.0	28 ± 2.1	30 25–37**
WR 2721 protected				
6000	1/12	8.5	29 ± 2.0	36 ± 1.6
7000	10/20	50.0	33 ± 1.5	31 ± 1.3
8000	14/22	64.0	40 ± 3.1	30 ± 1.4
9000	14/17	82.0	52 45–65**	33 30–35**

* 10 weeks without evidence of recurrence
** range

Results

Long term tumor control was achieved only with doses above 4000 rad in two fractions. The observation period for assessment of late recurrence was a minimum of six weeks after irradiation. Irradiated animals dying during this observation period with no evidence of local recurrence, were not considered to have long term tumor control, and were eliminated from the investigation.

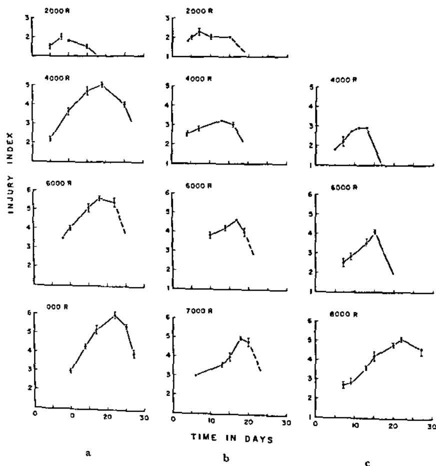


Fig 2 Variation of severity of the skin reaction with time for different doses of radiation Each point shows the mean and standard deviation based on 15 animals a) No drug b) Cysteamine c) WR 2721

Many of these died with pulmonary metastases Proportionate numbers of animals were excluded in three groups irradiated

Fig 1, summarizing the data in the Table, shows the per cent of tumors controlled related to the dose of radiation for unprotected animals and those protected with cysteamine or WR 2721 The protective effect was characterized by displacement of survival rates in the direction of higher doses The dose reduction factor for 50 per cent survival was calculated from

Table

Number of tumor-bearing animals irradiated without drugs (nonprotected), after Cysteamine (MEA protected), and after WR 2721 (WR-2721 protected) The time until recurrence or death is an average value with standard deviation Where numbers were small a range of time rather than the standard deviation is shown

Radiation dose (rad)	Controlled*		Time in days	
	No	%	Until recurrence	Recurrence until death
Nonprotected				
2500	0/8	0 0	12±1 8	21±2 1
3000	0/8	0 0	16±0 9	25±1 3
3500	0/8	0 0	20±1 2	25±1 6
4000	0/10	0 0	20±1 6	31±1 0
4500	1/12	8 3	20±1 5	29±1 4
5000	2/16	12 5	37±2 4	29+3 1
5500	7/15	46 7	42±4 4	30±2 6
6000	10/12	83 0		
7000	8/9	89 0		
MEA protected				
4500	0/11	0 0	15+1 2	31±2 1
5000	0/14	0 0	18±1 0	27±2 7
6000	0/10	0 0	20±0 8	30±4 1
7000	4/11	36 0	24±2 6	28±3 2
8000	4/7	57 0	28±2 1	30 25–37**
WR 2721 protected				
6000	1/12	8 5	29±2 0	36±1 6
7000	10/20	50 0	33±1 5	31±1 3
8000	14/22	64 0	40±3 1	30+1 4
9000	14/17	82 0	52 45–65**	33 30–35**

* 10 weeks without evidence of recurrence
** range

Results

Long term tumor control was achieved only with doses above 4 000 rad in two fractions The observation period for assessment of late recurrence was a minimum of six weeks after irradiation Irradiated animals dying during this observation period with no evidence of local recurrence, were not considered to have long term tumor control, and were eliminated from the investigation

gate the protective effect of normal tissues, but they quoted BACQ's (1954) earlier work, showing a protective effect of cysteamine in animals receiving whole body irradiation SCHWARTZ et coll using a mammary adenocarcinoma, showed little change in tumor growth rate comparing unprotected animals to animals protected by 2 mercaptoethylguanidine (MEG) Their demonstration of this drug s protective action was in its prolongation of survival time after 1 000 rad whole body irradiation YUHAS & STORER, using WR-2721, compared skin ulcerations to the TD50 of spontaneous mammary tumors of the C57 mouse They estimated a 15 per cent tumor protection based on end point dilution assays, and delay in time until the appearance of gross tumor The dose for production of 50 per cent skin ulceration was found to be 240 per cent greater in the protected animals

In the present investigation, radiation damage to the tumor cells has been measured by long term tumor control The end point was chosen to bring the investigation closer to clinical experience and because the TD50 following in vivo and in vitro irradiation has been established for this tumor (KALLMAN et coll 1967, 1968 VAN PUTTEN & KALLMAN 1968) This end point does not have the disadvantage of statistical variation found in end point dilution technique and animal survival times The dose reduction for the KHT tumor of 1 3 with cysteamine and 1 2 with WR 2721 was higher than the 1 15 obtained by YUHAS & STORER The difference between these two values probably reflects experimental differences, such as the end point selected, tumor type, and radiation regime

The numerical grading system of skin reactions has been shown to be effective in scoring radiation damage by both DENEKAMP et coll (1969) and VAN DEN BRENK (1969) The character of the progression of the acute skin reaction from an erythema to a necrosis is related to the dose given A change in the radiation dose affects both the acuteness and severity of the reaction, and the duration of the maximum reaction, whether it be desquamation or necrosis The protected animals show not only a diminished severity of skin reaction, but also a more rapid repair of the clinically demonstrable lesion

The intent of this research was to increase the therapeutic ratio The comparison of the reduction factors obtained from the acute skin reactions to the tumor protective effect of cysteamine gives a ratio of 1 7/1 3, yielding a 30 per cent advantage and for WR 2721 a ratio of 2 0/1 2, giving a 60 per cent advantage It seems possible, therefore, that systemic treatment with either protective agent used in the appropriate fractionation scheme can significantly improve the therapeutic ratio

Radioprotective drugs have been administered to humans in only limited situations, due to their high toxicity when used at therapeutic concentrations

$$\frac{\textit{dose for 50 per cent survival, unprotected}}{\text{dose for 50 per cent survival, protected}}$$

A reduction factor for cysteamine's protective effect on the tumor was calculated as 1.3 and for WR-2721 as 1.2. Another apparent dose-related factor was the time for the appearance of a recurrent tumor (Table). At a dose of 2 500 rad in two fractions, many of the tumors decreased only slightly in size and never were clinically absent. At doses greater than 5 000 rad in two fractions, recurrences appeared as late as 7 to 8 weeks after irradiation. In contrast, there was no apparent dose relationship in the time interval from recurrence of tumor *until death of the animal. It was observed that unirradiated control animals died* from massive local tumor with no pulmonary metastases, gross or microscopic. All animals irradiated who died with local recurrences also had pulmonary *metastases. Five to 10 per cent of these animals also had ipsilateral axillary* metastases. No other peripheral lymph node involvement was noted, nor were any abdominal metastases seen.

Fig. 2 describes the graded acute skin reactions related to time in days after the first of two irradiations. Fig. 2 a shows the reactions seen in the unprotected animals, Fig. 2 b the protective effect of cysteamine, Fig. 2 c the protective effect of WR-2721. The specific dose reduction factor for the acute skin reaction was computed by comparing the dose of radiation in protected and unprotected animals that produced only dry desquamation (grade 4 reaction) and moist desquamation (grade 5 reaction).

$$DRF_{DRY} = \frac{2450 \text{ rad (cysteamine)}}{1500 \text{ rad (unprotected)}} = 1.6$$

$$DRF_{DRY} = \frac{3000 \text{ rad (WR-2721)}}{1500 \text{ rad (unprotected)}} = 2.0$$

$$DRF_{MOIST} = \frac{3500 \text{ rad (cysteamine)}}{2000 \text{ rad (unprotected)}} = 1.8$$

$$DRF_{MOIST} = \frac{1000 \text{ rad (WR-2721)}}{2000 \text{ rad (unprotected)}} = 2.0$$

Discussion

The protective differential between normal and neoplastic tissues produced by various sulfhydryl compounds has been investigated by COHEN & COHEN (1959), SCHWARTZ et coll. (1961) and YUHAS & STORER (1969). COHEN & COHEN found minimal tumor protection of a C3H mammary tumor when irradiated in combination with systematically administered cysteamine. They did not investi-

geschutzte Tiere 6900 rad benotigen Der Schutzeffekt dieser Substanzen auf die akuten Hautreaktionen wurde bei gleicher fraktionierter Bestrahlung untersucht Der therapeutische Index, d h das Verhaltnis der Tumorlethaldosis und der Gewebtoleranzdosis stieg für Cysteamin um 30 Prozent und fur WR 2721 um 60 Prozent

RÉSUMÉ

Les agents radio protecteurs cystéamine et WR 2721, associés au traitement par les radiations ont augmente le taux de succès therapeutique dans le traitement du sarcome KHT greffe sur la cuisse de souris C3H/HeJ La guérison de la tumeur chez 50 pour cent des animaux non proteges a necessité 5 600 rad en deux fractions, alors que les animaux protéges par le WR 2721 ont necessite 6 900 rad L'action protectrice de ces agents sur les reactions cutanées aigues a ete etudiee par des series d irradiations fractionnees semblables Le rapport therapeutique, rapport entre la dose lethale tumorale et la dose de tolerance des tissues, a ete trouve augmente de 30 pour cent pour la cysteamine et 60 pour cent pour le WR-2721

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SCHWARTZ E E, SHAPIRO B and KOLLMAN G Selective chemical protection [illegible] in tumor [illegible]

VAN DEN B[illegible] to skin [illegible]

VAN PUTTE[illegible] KALLMAN R F Oxygenation stat[illegible] fract[illegible]

YUH[illegible] J[illegible]

BACQ et coll (1952), administered cysteamine in the treatment of leukemic patients, with doses from 100 mg to 500 mg daily, for as long as 30 days, and found a low toxicity. They also noted little change in the clinical course of the disease at these dose levels COURT-BROWN (1955) used cysteamine in an attempt to minimize the acute radiation syndrome in patients receiving large integral doses of irradiation He found no ameliorating effect at drug levels tolerated by the patients Successful protection in primates receiving whole body irradiation was demonstrated by MELVILLE et coll, in administering a combination of AET and L-cysteine

Early investigators felt that radiation protective agents would be applicable in the treatment of individuals receiving acute accidental radiation exposure To demonstrate a protective effect, the drug must be administered before irradiation This precludes its use in any situation but that of planned exposure This condition most commonly arises in the course of radiation therapy

Acknowledgements

The authors wish to acknowledge with gratitude, the technical assistance of H Kowta who also prepared the figures The authors also express their appreciation to M L Meurk and to Dr K McCormack, for their helpful discussions and comments during preparation of the manuscript, and to Dr J M Vaeth without whose encouragement and wise counsel this investigation would not have been possible

The investigation was supported in part by Public Health Service Research Grant CAO5203 from the National Cancer Institute and an American Cancer Society Clinical Fellowship

SUMMARY

The radioprotective agents cysteamine and WR 2721 combined with radiation therapy improved the therapeutic ratio in treatment of the KHT sarcoma implanted in the thigh of C3H/HeJ mice Tumor control in 50 per cent of the unprotected animals required 5 600 rad in two fractions while the cysteamine protected animals required 7 500 rad and WR 2721 protected animals 6 900 rad The protective action of the agents on the acute skin reaction was investigated through similar fractionated courses of irradiation The therapeutic ratio the relationship of the tumor lethal dose to the tissue tolerance dose was found to have been increased by 30 per cent for cysteamine and 60 per cent for WR 2721

ZUSAMMENFASSUNG

Die Strahlenschutzsubstanzen Cysteamin und WR 2721 verbesserten in der Kombination mit der Strahlentherapie das therapeutische Verhaltnis bei der Behandlung des KHT Sarkoms das in die Flanke von C3H/HeJ Mausen implantiert worden war Um die Tumor kontrolle von 50 Prozent der nich geschutzten Tiere zu erreichen wurden 5 600 rad in zwei Fraktionen benotigt, wahrend durch Cysteamin geschutzte Tiere 7 500 rad und WR 2721

vestigation is an attempt to characterise the lymphocytes infiltrating carcinoma of the breast as regards their capacity to be activated by phytohaemagglutinin in vitro

Methods The primary diagnosis of mammary carcinoma is usually made by fine needle aspiration biopsy (FRANZEN & ZAJICEK 1968) Four consecutive cases with cytologic signs of extensive lymphocyte infiltration in the tumour were selected A piece of the tumour was dissected out within 30 minutes of removal of the breast Cells from the neoplasms were obtained by gently cutting them into thin slices in Eagle's minimal essential medium (MEM) supplemented by Earles salts The cells released into the medium were then sedimented by centrifugation and resuspended in MEM, cell number and viability were determined in a Burker chamber after conventional Trypan blue staining The proportion of lymphoid cells was first assessed by counting in the chamber, the tumour cells were usually readily distinguishable from the lymphocytic cells by their larger size The proportion of Trypan blue positive cells varied between 15 and 30 per cent Most of the dead cells had the appearance of tumour cells To verify further the frequency of lymphocytic cells, smear preparations were made from the cell suspensions, between 200 and 300 cells being scored after Giemsa staining The tumour cells appeared larger than the lymphoid cells and had pale stained nuclei whereas the latter were darker In the few cases of doubt as to whether a cell was lymphoid or not it was classified as a tumour cell

Venous blood obtained from the patients about an hour before operation was defibrinated by gentle shaking in a beaker containing glass pearls Three per cent gelatin dissolved in Hanks Tris buffer in a v/v ratio of 3 1 (blood gelatin solution) was added and the erythrocytes were allowed to sediment at unit gravity for 45 *minutes* at 37° C, the supernatants were then collected, washed h [illegible] lymphoid cells was [illegible] The frequency [illegible] non lymphoid cells varied between 10 and 30 per cent The viability of the cells, as determined after Trypan blue staining, exceeded 90 per cent

Phytohaemagglutinin reactivity of cells in vitro Culture conditions and measurements of isotope uptake by the cells has been described previously (EINHORN et coll 1970) Various numbers of viable nucleated cells were suspended in 1 0 ml MEM containing 150 units of penicillin and 150 µg streptomycin/ml supplemented with 10 per cent serum from human donors of blood groups AB, Rh+ (the serum was previously heated to 56° C for 30 minutes and stored at —20° C before use) The cells were kept in 15 ml screw-cap glass tubes with

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LYMPHOID CELLS IN CARCINOMA OF THE BREAST

Failure of response to phytohaemagglutinin in vitro

H BLOMGREN, ULLA GLAS, S FRANZEN and P -O GRANBERG

The peripheral lymphoid organs of vertebrates contain two major classes of immunocompetent lymphoid cells One of these is dependent on the thymus (T-cells) and the other on the bursa of Fabricius in avians (B cells) (ROITT et coll 1969) and its possible analogue in mammals (COOPER et coll 1966) Investigations into the survival of allogenic and xenogenic grafts in thymectomized individuals indicate that T-cells are necessary for graft nonacceptance (MILLER & OSOBA 1967) Experimental support also exists for the view that the presence of T-cells is important for the rejection of certain syngenic tumours (MILLER et coll 1964, GRANT et coll 1965) The role of B cells during graft rejection is less clear Experimental data indicate that thymus-independent lymphocytes may lyse cells in vitro, being coated with small amounts of antibodies directed against specific membrane antigens (HARDIN et coll 1971, BOXEL et coll 1972)

The prognosis is considered to be favourable in different types of malignant growths with histologic evidence of infiltration of lymphocytes (HULTBORN & TORNBERG 1960, CUTLER et coll 1969) The invasion may be interpreted as a sign of active immunological reaction against the tumour The present in-

Submitted for publication 16 November 1972

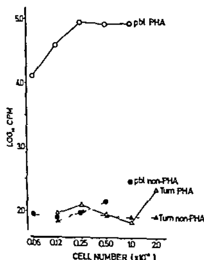


Fig 1 Case 1 Incorporation of ^{14}C-thymidine in vitro expressed as $\log_{10}$ counts per minute by cells obtained from a breast carcinoma and autochthonous peripheral blood lymphocytes Various numbers of cells were cultured in 1 0 ml medium in the presence or absence of phytohaemagglutinin 25 to 33 per cent of the cells obtained from the tumour were of the lymphoid type Pbl stands for peripheral blood lymphocytes and Tum for cells obtained from tumour

obtained demonstrated that the lymphoid cells infiltrating the tumour exhibit an extremely low capacity to respond to phytohaemagglutinin as compared to autochthonous blood lymphocytes

Case reports

Case 1 Female aged 29 Radical mastectomy performed for a lump in the right breast detected a month previously and after delivery of a third child, the tumour had a diameter of about 8 cm with spread to the axillary lymph nodes Histology medullary type of carcinoma of the breast with extensive lymphocytic infiltration The peripheral blood lymphocytes exhibited an increase in isotope uptake in response to phytohaemagglutinin by increasing the number of cultured cells from about 6×10^4 up to 2.5×10^5 A further increase in cell concentration failed to result in an increased ^{14}C thymidine uptake The cells obtained from the tumour exhibited only slight if any stimulation by phytohaemagglutinin Some 30 per cent of the cultured cells from the tumour were of the lymphoid type (Table) hence 2×10^6 cultured cells from the tumour would contain lymphocytes in an amount equivalent to cultures with 0.5×10^6 blood lymphocytes, the difference in thymidine uptake by these two cell cultures being at least a hundredfold

Case 2 Female aged 44, with three weeks history of a lump in the right breast, this was removed by mastectomy The mass measured about 4.5 cm in diameter, metastases were evident in the lymph nodes of the right axilla Histology med ll extensive lymphocy ...
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Table

Frequency of lymphoid cells in suspensions prepared from the tumours. The calculations were made after differential counts in a Burker chamber of living cells and on smear preparations with Giemsa staining. A tumour of the breast and an axillary metastasis were tested in Case 2

Case	Percentage of lymphocytic cells	
	Smear preparation	Burker chamber
1	33	25
2 Tumour	29	55
Metastasis	78	74
3	23	20
4	30	24

a diameter of 15 mm and a rounded bottom. Each cell concentration was set up in quadruplicate and half of the cultures received phytohaemagglutinin (Bacto-Phytohaemagglutinin M, Difco Lab., Detroit, Mich, USA) at a final concentration of three per cent. After three days of culture at 37° C in a humified CO_2 atmosphere of five per cent each cell culture received 0.4 μCi ^{14}C-thymidine (50 mCi/mM, The radiochemical Centre, Amersham, England) in 0.1 ml MEM. The cultures were terminated twenty-four hours later and the cells washed in balanced salt solution by centrifugation before being precipitated in 5 % trichloroacetic acid. The precipitates were then dissolved in soluene, transferred to Packard scintillation vials containing scintillation fluid and the radioactivities measured in a Packard scintillation counter model 3380.

The $\log_{10}$ mean isotope incorporations of the duplicate cultures, expressed as counts per minute, were calculated. The values for the duplicate cultures that had received phytohaemagglutinin did not differ more than 0.2 $\log_{10}$ units, whereas cultures without this addition sometimes varied by a factor of up to 0.35 $\log_{10}$ units.

Results

Most of the lymphoid cells appeared small and non-blast transformed, occasional plasma cells were present in all tumour smears tested. The results of the differential counts are presented in the Table, some 20 to 80 per cent of the cells were lymphocytic and reasonable agreement was evident between the two techniques applied for differentiating the cells.

Lymphocytes obtained from the blood and tumour of the four different patients were tested on the same day and under identical conditions. The results

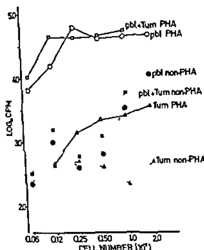


Fig 4 Case 4 Same experimental method 24 to 30 per cent of the tumour cells obtained were of the lymphoid type

Discussion

The present investigation was aimed at the in vitro phytohaemagglutinin reactivity of lymphocytes infiltrating carcinoma of the breast in human subjects Histology revealed that in two cases the tumours exhibited completely, and the other 2 cases partly the morphology typical of medullary carcinoma This condition is known often to exhibit extensive infiltration by lymphocytes and have a relatively favourable prognosis compared to other types of carcinoma of the breast (MORE & FOOTE 1949, BLOOM 1970)

The lymphocytes infiltrating the tumour may represent cells in a stage of active sensitization against antigenically foreign structures on the malignant cells rendering them a specific cytotoxic potential It appears that T cells may be sensitized against alloantigens thereby achieving a cytotoxic potential against the cells bearing the antigens used for the purpose (CEROTTINI et coll 1970, BLOMGREN et coll 1970, BLOMGREN & SVEDMYR 1971) The specific sensitizing step may occur in the absence of B cells (GOLSTEIN et coll 1972) and evidence also exists that the actual killing of the target cells by the sensitized lymphocytes may take place in the absence of B cells (GOLSTEIN et coll 1972) These observations suggest that it might be assumed that the lymphocytes invading neoplasms may be of thymic origin Since phytohaemagglutinin is considered to have a high specific mitogeneic effect on T cells (RIEKE 1966, GREAVES et coll 1968 BLOMGREN & SVEDMYR 1971) an attempt was made to compare the phytohaemagglutinin responsiveness of lymphocytes infiltrating a tumour and autochthonous blood lymphocytes in vitro An extremely poor mitogeneic effect in the lympho-

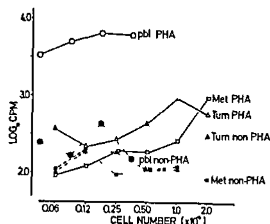


Fig 2 Case 2 In addition to cells obtained from the tumour of the breast and the peripheral blood an axillary metastasis was also tested 29 to 55 per cent of the tumour cells and 74 to 78 per cent of the metastasis cells were of the lymphoid type (Symbols as in Fig 1)

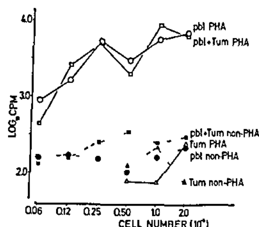


Fig 3 Case 3 Cultures were also set up with varying numbers of peripheral blood lymphocytes admixed with a constant number of tumour cells (0.5×10^6) 20 to 23 per cent of the latter were of the lymphoid type (Symbols as in Fig 1)

than 70 per cent lymphocytic cells) a higher thymidine incorporation in response to phytohaemagglutinin was obtained by the smallest number of cultured blood lymphocytes i e 6×10^4 than the greatest number of cultured cells from both tumours or 2×10^5

Case 3 Female, aged 47 Radical mastectomy for 2.5 cm lump in right breast said to have been present for 2 weeks no metastases Histology Poorly differentiated carcinoma partly scirrhous partly medullary and partly comedo Extensive lymphocytic infiltration The comparison of phytohaemagglutinin responsiveness between blood and tumour lymphocytes in Cases 1 and 2 indicated an extremely low reactivity of those obtained from the tumour To test the possibility that this difference might have been due to the contaminating malignant cells control cultures were included containing various numbers of blood lymphocytes admixed with a fixed number of cells from the tumour The data obtained in this case indicated that the cells from the tumour failed to inhibit the phytohaemagglutinin responsiveness of the peripheral blood lymphocytes and confirmed the low responsiveness of lymphocytes from the tumour

Case 4 Female aged 51 Radical mastectomy following three weeks history of a lump measuring about 1.75 cm in diameter No known metastases Histology Predominantly poorly differentiated mammary duct carcinoma with areas of comedo type and medullary carcinoma Extensive lymphocytic infiltration The cells from this tumour exhibited the highest phytohaemagglutinin response of those tested However comparison between the response of blood lymphocytes and an equivalent number of lymphocytes from the tumour confirmed the low reactivity of the latter cells In agreement with the results from Case 3 the admixture of 0.5×10^6 cells from the tumour with the blood lymphocytes failed to decrease their responsiveness

ZUSAMMENFASSUNG

Die Lymphozyten, die Krebsgewebe der menschlichen Brust infiltrieren, und die autochtonen Blutlymphozyten wurden hinsichtlich ihrer Stimulation in vitro durch Phythamagglutinin untersucht Es wird uber vier Falle berichtet, in allen diesen Fallen waren die infiltrierenden Lymphozyten geringfugig stimuliert, während die Blutlymphozyten mit einem hochgradig gesteigerten Einbau von radioaktiv-gezeichnetem Thymidin reagierten Die Resultate werden dahingehend interpretiert, dass die Tumoren nahezu selektiv die nicht-Thymus abhangigen Lymphozyten attrahiert hatten

RÉSUMÉ

Les auteurs ont compare l effet de la stimulation in vitro par la phytohemagglutinine sur les lymphocytes envahissant le cancer du sein de la femme et sur les lymphocytes sanguins autochtones Ils presentent 4 cas, dans tous ces cas les lymphocytes infiltrants etaient peu stimules alors que les lymphocytes sanguins repondaient à la stimulation par une incorporation tres augmentee de la thymidine marquee par un isotope Ces résultats ont eté interpretes comme indiquant que les tumeurs attiraient presque selectivement les lymphocytes qui ne dependent pas du thymus

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killing of allogeneic ta [illegible]

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cytotoxic cells Europ J Immunol 2 (1972), 498

cytes invading the former was evident in the 4 cases investigated Blood lymphocytes from the same cases exhibited high thymidine incorporations in response to this mitogen This may indicate that cells other than T-cells had almost selectively invaded the masses Another explanation may be that they were of thymic origin but unresponsive to phytohaemagglutinin due to an active sensitization continuing against tumour associated antigens The lymphocytes infiltrating the malignant tissue were, however, small in size with scanty cytoplasm and darkly staining nuclei, a high thymidine incorporation in vitro by the cells in the absence of phytohaemagglutinin, that might have been expected, was not observed Another possibility may be that the malignant cells inhibit the phytohaemagglutinin response of the lymphocytes, this explanation seems less likely since the admixture of the cells to cultures containing blood lymphocytes failed to change the responsiveness

It would appear that the majority of lymphoid cells infiltrating breast carcinoma are other than T-lymphocytes In support of this view is the preliminary finding that such lymphocytes fail to bind sheep erythrocytes in vitro, which seems to be a specific characteristic of human T-lymphocytes (JONDAL et coll 1972)

The results fail also to establish that most of the lymphocytes infiltrating the tumours are B-lymphocytes, although cells with a typical morphology of plasma cells were present in the growths It is possible that these cells represent a cell population produced in the bone marrow (OSMOND & EVERETT 1964) not processed by the bursa equivalent It is conceivable that T-lymphocytes exist in the malignant tissue and react against foreign antigens and thereby stimulating lymphocytes from bone marrow to invade the lesion Such a massive migration of these lymphocytes has been observed in the delayed type of skin reactions known to be thymus dependent (LUBAROFF & WAKSMAN 1968)

Acknowledgements

The authors take this opportunity of thanking Prof J Einhorn J Wasserman and Prof G Moberger for their support Mrs Britta Maria Berg is also thanked for technical assistance This investigation was supported by grants from Gustaf V Jubileumsfond

SUMMARY

Lymphocytes invading carcinoma of the human breast and autochthonous blood lymphocytes were compared regarding their stimulation in vitro by phytohaemagglutinin Four cases are reported, in all these the infiltrating lymphocytes were poorly stimulated whereas the blood lymphocytes responded by a highly increased incorporation of isotope labelled thymidine The results were interpreted as indicating that the tumours had almost selectively attracted the nonthymus dependent lymphocytes

ZUSAMMENFASSUNG

Die Lymphozyten die Krebsgewebe der menschlichen Brust infiltrieren, und die autochtonen Blutlymphozyten wurden hinsichtlich ihrer Stimulation in vitro durch Phythamagglutinin untersucht Es wird uber vier Falle berichtet in allen diesen Fallen waren die infiltrierenden Lymphozyten geringfugig stimuliert wahrend die Blutlymphozyten mit einem hochgradig gesteigerten Einbau von radioaktiv gezeichnetem Thymidin reagierten Die Resultate werden dahingehend interpretiert, dass die Tumoren nahezu selektiv die nicht Thymus abhangigen Lymphozyten attrahiert hatten

RÉSUMÉ

Les auteurs ont compare l effet de la stimulation in vitro par la phytohemagglutinine sur les lymphocytes envahissant le cancer du sein de la femme et sur les lymphocytes sanguins autochtones Ils presentent 4 cas dans tous ces cas les lymphocytes infiltrants etaient peu stimules alors que les lymphocytes sanguins repondaient a la stimulation par une incorporation tres augmentee de la thymidine marquee par un isotope Ces resultats ont ete interpretes comme indiquant que les tumeurs attiraient presque selectivement les lymphocytes qui ne dependent pas du thymus

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FROM RADIUMHEMMET (DIRECTOR PROF J EINHORN), 104 01 STOCKHOLM, AND STATENS BAKTERIOLOGISKA LABORATORIUM DEPARTMENT OF IMMUNOLOGY (DIRECTOR PROF A FAGRAEUS) 105 21 STOCKHOLM, SWEDEN

THYROID ANTIBODIES AND HYPOTHYROIDISM IN ^{131}I THERAPY FOR HYPERTHYROIDISM

G LUNDELL and J JONSSON

Seven to twelve per cent of patients with hyperthyroidism treated with ^{131}I develop hypothyroidism within a year (WERNER et coll 1957, CASSIDY & ASTWOOD 1959 MACGREGOR 1960 BELING & EINHORN 1961) and at seven to ten years 25 to 30 per cent are hypothyroid (BELING & EINHORN 1961, GREEN & WILSON 1964 BECKER et coll 1971) There is evidence that ^{131}I treatment is followed by an autoimmune reaction to the irradiated tissue (EINHORN et coll 1969 1971) as manifested by an increase in thyroid cytoplasmic antibodies in both hyperthyroid (BUCHANAN et coll 1962 O GORMAN et coll 1964, IRVINE 1964 EINHORN et coll 1965 JONSSON et coll 1968) and euthyroid patients (EINHORN et coll 1966) this seems to be due to the treatment and not to the reduction in gland size or to a fall in the metabolic rate (HJORT & MOGENSEN 1962, EINHORN et coll 1965) An increase in antibodies to the [illegible]

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Submitted for publication 13 October 1972

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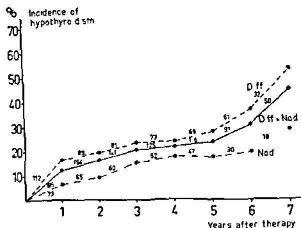


Fig 2 Frequency of hypothyroidism related to the time elapsing after ^{131}I therapy for hyperthyroidism Nodular or diffuse goitre or both The number of patients available for follow up at the beginning of each period appears in the diagram Broken lines denote less than 20 observations

Antibodies to thyroglobulin were determined by the Boyden passive hemagglutination test with tanned red cells (TC) (DERRIEN et coll 1948, WITEBSKY & ROSE 1956) Patients with titres $< 1/20$ for antibodies to thyroglobulin were designated as negative and those with titres $\geqslant 1/20$ positive The antibodies to cytoplasmic thyroid antigen (cytoplasmic fluorescence, CF) were determined by Coon's indirect immunofluorescence technique (ROITT & DONIACH 1958, HOLBOROW et coll 1959)

The methods for calculation of the cumulative frequency of hypothyroidism have been described elsewhere (BELING & EINHORN) Except for Fig 2, the graphs were discontinued at the end of the fifth year after treatment because the number of patients was then too small to serve for evaluation The patients were sometimes few even after a shorter period, where less than 20 observations were available the curves have been drawn with broken lines The statistical methods used were the chi square test and Student's t-test

Results

The cumulative frequency of hypothyroidism after ^{131}I therapy for hyperthyroidism in the different groups of patients appears in Figs 2, 3 and 4

Of the 73 patients who before treatment had demonstrable antibodies to cytoplasmic thyroid antigen 52 per cent developed hypothyro dism during the period of observation, against 21 per cent of 115 patients without these antibodies, the difference is statistically significant ($p < 0.001$, Table 1) Both early ($p < 0.01$) and late hypothyroidism ($p < 0.01$) were evident significantly more often in the patients with demonstrable antibodies to cytoplasmic thyroid

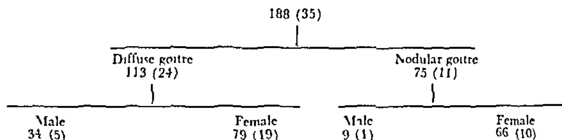


Fig 1 Distribution of patients before ^{131}I therapy The figures in parentheses denote those previously undergoing surgical treatment

Patients treated with ^{131}I for hyperthyroidism displayed a correlation between early hypothyroidism (*developing within one year after the therapy*) and the presence of thyroid antibodies after therapy (EINHORN et coll 1965)

The intention of this investigation was to ascertain whether any correlation exists between thyroid antibodies before and after ^{131}I therapy for hyperthyroidism and the development of hypothyroidism

Material A total of 188 patients given ^{131}I therapy for hyperthyroidism during the period 1963—69 were examined for the occurrence of antibodies to thyroglobulin and cytoplasmic thyroid antigen before, and similarly controlled at regular intervals after therapy The principles of this treatment have been previously reported (LARSSON 1955, BELING & EINHORN 1961) The series consisted of 43 men and 145 women aged 23 to 83 (mean 55 years) at the time of the first treatment There were *113* with diffuse goitre or without palpable enlargement of the thyroid, and 75 patients with nodular goitre Thirty-five patients had had previous surgical treatment for hyperthyroidism (Fig 1)

Methods The patients were controlled at intervals of 2 to 4 months during the first year after treatment, and thereafter every year until they developed hypothyroidism or to the end of the investigation Three patients could not be *followed in accordance with this scheme for one year, and an additional* 31 patients could not be controlled for two years The number of patients available appears along the curves in Fig 2

The patients were classed as *hypothyroid on the basis of their clinical condition* supported by the determinations of protein bound iodine, serum cholesterol, the T3-resin and radioiodine tracer tests A determination of protein bound ^{131}I was sometimes made before therapy The tendency for a rise in the frequency of *hypothyroidism* during the last year of the investigation (Fig 2) may well have been due to the authors' efforts to examine as many of the patients as possible within its last 6 months

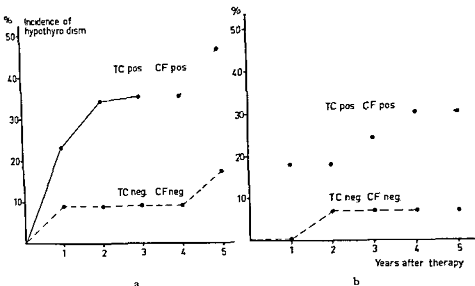


Fig 4 Frequency of hypothyroidism related to the time elapsing after ^{131}I therapy for hyperthyroidism in relation to antibodies to cytoplasmic thyroid antigen (CF) and thyroglobulin (TC) before therapy a) diffuse goitre b) nodular goitre

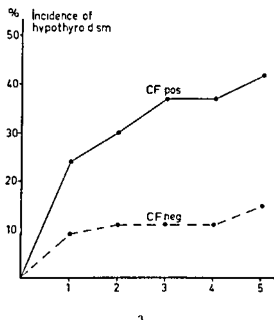
these antibodies (Table 2) No significant correlation was recorded for different titre levels $\geq$ 1/20 of antibodies to thyroglobulin and the frequency of hypothyroidism (Table 3) Of 52 patients who before treatment had demonstrable antibodies to both cytoplasmic thyroid antigen and thyroglobulin, 56 per cent developed hypothyroidism, against 16 per cent of the 76 patients without any such antibodies ($p < 0.001$, Table 4)

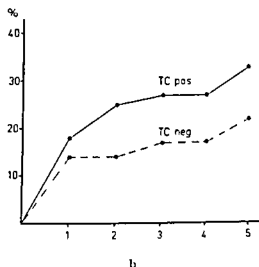
As in previously published series, patients with diffuse goitre or without palpable enlargement of the thyroid gland developed hypothyroidism more often than those with nodular goitre (Table 5)

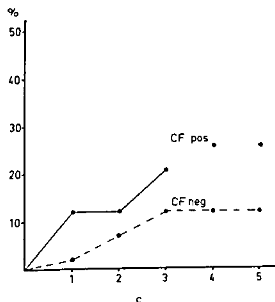
Patients with positive titres of antibodies to thyroglobulin or demonstrable antibodies to cytoplasmic thyroid antigen before radioiodine therapy displayed a higher frequency of hypothyroidism after the treatment than those without these antibodies, this applied both to those with diffuse and to those with nodular goitre (Figs 3, 4) The lowest frequency of hypothyroidism occurred

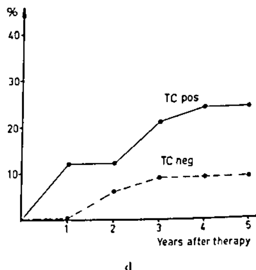
treatment (Fig 4 a)

As in previously published series there was a significant increase in antibodies to cytoplasmic thyroid antigen 2 to 12 months after treatment ($p < 0.05$) but









...ie time elapsing after ^{131}I therapy for hyper...
...thyroid antigen (CF a c) and thyroglobulin
...ic antigen diffuse goitre b) thyroglobulin
diffuse goitre c) thyroid cytoplasmic antigen nodular goitre d) thyroglobulin nodular goitre

antigen before treatment than in those without such antibodies (Table 1) Hypothyroidism was also present more often in those with antibody titres to thyroglobulin $\geqslant 1/20$ before treatment ($p < 0.001$) than in patients without

Table 4

Antibodies to cytoplasmic thyroid antigen and thyroglobulin before ^{131}I therapy against posttherapy hypothyroidism

	No. of patients	Hypothyroid, per cent of total		
		≤ 1 year	> 1 year	all hypothyroid
Both types of antibodies				
demonstrable	52	23	42	56
Non-demonstrable	76	5	9	16
P		< 0.01	< 0.001	< 0.001

Table 5

Type of goitre against posttherapy hypothyroidism

	No. of patients	Hypothyroid, per cent of total		
		≤ 1 year	> 1 year	all hypothyroid
Diffuse goitre	113	16	29	41
Nodular goitre	75	5	17	21
P		< 0.05	< 0.05	< 0.01

developed early hypothyroidism than those with no such antibodies either before or after therapy, this did not occur with antibodies to thyroglobulin (Table 7).

Discussion

As previously reported a considerable number of patients in the present material developed hypothyroidism after ^{131}I therapy for hyperthyroidism (Fig. 2). The relatively high frequency of hypothyroidism in this series, even higher than in earlier material from this department (BELING & EINHORN), may be due to the fact that during the period of this investigation several physicians were involved in the treatment; this has been shown to increase the frequency of hypothyroidism (BELING & EINHORN).

After surgery for hyperthyroidism, a correlation was found between the presence of antibodies to thyroid antigen before the treatment and the development of hypothyroidism (HJORT & MOGENSEN 1962); the same occurred for ^{131}I treatment of hyperthyroidism in the present material. Patients in whom demonstrable antibodies to thyroid antigen were present before ^{131}I treatment

Table 1

Antibodies to cytoplasmic thyroid antigen before ^{131}I therapy against posttherapy hypothyroidism

	No of patients	Hypothyroid, per cent of total		
		< 1 year	> 1 year	all hypothyroid
Antibodies demonstrable	73	21	40	52
Antibodies non-demonstrable	115	6	16	21
p		< 0.01	< 0.01	< 0.001

Table 2

Antibodies to thyroglobulin before ^{131}I therapy against posttherapy hypothyroidism

	No of patients	Hypothyroid, per cent of total		
		< 1 year	> 1 year	all hypothyroid
Titre > 1/20	90	16	34	44
Titre < 1/20	98	8	16	23
p			0.01	< 0.001

Table 3

Antibodies to thyroglobulin before ^{131}I therapy against posttherapy hypothyroidism Relation to different titre levels > 1/20

	No of patients	Hypothyroid per cent of total					
		< 1 year		> 1 year		all hypothyroid	
		No	%	No	%	No	%
Titre 1/20–1/100	30	4	13	6	23	10	33
1/200–1/800	26	3	11	9	39	12	46
> 1/1600	34	6	17	11	39	17	50
p			–		–		–

no increase in antibodies to thyroglobulin (Table 6) No correlation between this increase in the thyroid antibodies after radiation therapy and the development of hypothyroidism was recorded (Table 6) However, patients with demonstrable antibodies to cytoplasmic thyroid antigen only after treatment, more often

therapy as compared to pre-therapy values (BUCHANAN et coll 1962, O'GORMAN et coll 1964, IRVINE 1964, EINHORN et coll 1965) (Table 6) Since a correlation between pre- and post-therapy values in the same patient is to be expected, it was not surprising that correlation also existed between the presence of thyroid antibodies 2 to 12 months after therapy and the development of hypothyroidism (EINHORN et coll 1965) No correlation between the increase in thyroid antibodies after the treatment and the development of hypothyroidism was, however, apparent

The increasing frequency of hypothyroidism following ^{131}I therapy for hyperthyroidism may have different explanations One of these is that the continuing decrease in thyroid function may be due to an immunologic reaction that was present before the treatment, or induced by it In favour of this explanation is 1) the correlation between the presence of antibodies to thyroid antigen before ^{131}I therapy and the subsequent development of hypothyroidism, 2) the post-therapy increase in antibodies to cytoplasmic thyroid antigen and 3) the fact that patients with demonstrable antibodies to cytoplasmic thyroid antigen after, but not before treatment more often developed early hypothyroidism than those not having these antibodies at any stage

Against this explanation is that no correlation was observed between the increase in thyroid antibodies after the therapy and the development of hypothyroidism for the series as a whole

It is possible that the frequency of hypothyroidism after ^{131}I therapy may be decreased by selecting the appropriate method of treatment and possibly a suitable dose level for the individual patient as well This level of antibodies to thyroid antigen before treatment may then be one of the valuable selection factors, as may also the type of goitre—nodular or diffuse Five years after treatment only 7 per cent of patients of this material with nodular goitre and no demonstrable antibodies developed hypothyroidism, this was against 46 per cent of those with diffuse or nonpalpable goitre and demonstrable antibodies to both cytoplasmic thyroid antigen and thyroglobulin It should, however, be kept in mind that after *surgical* treatment for hyperthyroidism a frequency of hypothyroidism of 25 per cent has also been reported in a long-term follow-up investigation (BECKER et coll 1971)

SUMMARY

Correlation is reported between the presence of thyroid antibodies before therapy and the development of hypothyroidism following it in a group of 188 patients given ^{131}I for hyperthyroidism Of the patients without antibodies to cytoplasmic thyroid antigen before treatment those who had such antibodies after treatment more often developed hypothyroidism within a year than those without antibodies either before or after the treatment

Table 6

Change in thyroid antibodies after ^{131}I therapy against posttherapy hypothyroidism

	No of patients	Hypothyroid, per cent of total		
		< 1 year	> 1 year	all hypothyroid
Antibodies to cytoplasmic thyroid antigen				
Increased	52	17	28	40
Unchanged	101	10	21	29
Decreased	27	11	33	41
p	< 0.05*	—	—	—
Antibodies to thyroglobulin				
Increased	16	13	28	37
Unchanged	87	15	19	31
Decreased	47	6	32	36
p	—		—	

* refers to number of patients with an increase in antibodies to cytoplasmic thyroid antigen

Table 7

Patients with no demonstrable antibodies to cytoplasmic thyroid antigen or thyroglobulin before therapy. Increase in antibodies to thyroid antigen 2 to 12 months after ^{131}I therapy against posttherapy hypothyroidism

	No of patients	Hypothyroidism per cent of total		
		< 1 year	> 1 year	all hypothyroid
Antibodies to cytoplasmic thyroid antigen				
Increased	36	14	19	31
Unchanged	73	3	15	18
p		< 0.05		
Antibodies to thyroglobulin				
Increased	14	0	14	14
Unchanged	81	11	17	26
p			—	—

developed both early and late hypothyroidism more often than those without these antibodies (Tables 1, 2, 3, 4, Figs 2, 3, 4) In patients with no antibodies to cytoplasmic thyroid antigen before treatment, hypothyroidism appeared to occur more often in those who developed these antibodies after treatment than in those who remained without antibodies also after treatment (Table 7) Antibodies to cytoplasmic thyroid antigen increased 2 to 12 months after ^{131}I

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ZUSAMMENFASSUNG

Ein Zusammenhang zwischen dem Vorkommen von Thyreoideaantikörpern vor der Therapie und der Entwicklung eines Hypothyreodismus nach Gabe von ^{131}I wegen einer Thyreoideaüberfunktion wird bei einer Gruppe von 188 Patienten beschrieben. Von den Patienten ohne Antikörper gegen Protoplasma-Thyreoideaantigen vor der Behandlung entwickelten diejenigen, die solche Antikörper nach der Behandlung hatten, häufiger Hypothyreoidismus innerhalb eines Jahres als diejenigen die keine Antikörper weder vor noch nach der Behandlung hatten.

RÉSUMÉ

Les auteurs ont trouvé une relation entre la présence d'anticorps thyroïdiens avant le traitement et l'apparition d'un hypothyroïdisme après le traitement sur un groupe de 188 malades traités par ^{131}I pour hyperthyroïdisme. Parmi les malades qui n'avaient pas d'anticorps à l'antigène cytoplasmique thyroïdien avant le traitement, ceux qui avaient de tels anticorps après le traitement ont constitué un hypothyroïdisme dans l'année qui a suivi le traitement plus souvent que les malades qui n'avaient d'anticorps ni avant ni après le traitement.

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fixed to an operating table after being anaesthetized with 25 mg/kg sodium pentobarbital Both animals species were kept without food for 24 hours prior to the experiments

Partial body irradiation was performed by covering the non irradiated parts of the body with 4 mm lead, and employing a Philips Muller MG 300 apparatus at 200 kV, 15 mA, and 0 5 mm Cu and 0 5 mm Al filtration The dose rate varied with the focus to object distance In rabbits the average dose rate as measured in cadavers by a Philips Universal dosimeter was 45 rad/min and in mice amounted to 74 rad/min for all regions irradiated To obtain platelet irradiation both carotid arteries of the rabbits were bared for 3 cm, a piece of 4 mm lead measuring 5 cm × 3 cm was then placed underneath and the whole animal covered by 4 mm lead with a 3 cm × 3 cm hole above the carotids The dose rate measured was 700 rad/min a value which was obtained with the 0 5 mm Al filter only

The diameter of the carotid arteries was estimated to find the dose received by the circulating blood and the blood volume exposed to the roentgen beam was calculated Assuming the blood content of the rabbits to be 52 ml/kg body weight (SCHILDT & SCHILDT 1963), and recognizing that the exposure time in the experiment was 2 1/2 h the total roentgen dose received by the whole blood was calculated according to the equation $D_t = \frac{V_c}{V} \times t \times D$, where D_t = total dose

V = volume of the exposed carotid part in ml
V = total blood volume in ml
t = exposure time in s
D_s = radiation dose per s

Venous blood in 4 5 ml samples from the ear for the first three samples and from one of the carotid arteries for the next three samples were collected in plastic tubes containing 0 5 *ml* EDTA 5 % The first two samples were taken prior to irradiation Blood sampling from the mouse was executed by orbita extraction, 0 8 ml blood being collected into 0 2 ml EDTA 2 5 % in physiologic saline All samples were kept at 4° C and spun at 1 250 g in a cooled centrifuge for 10 min Plasma was withdrawn by aspiration and respun at 1 250 g for 30 min Two samples of the platelet free murine plasma were pooled All samples were analysed for their 5 HT content by the method of ASHCROFT et coll (1963) Hemolytic samples were discarded To prevent breakdown of the blood 5 HT the animals were intraperitoneally injected 30 min before the start of the experiment with either 10 mg/kg tranylcypromine or 10 mg/kg iproniazide, both are well known inhibitors of the enzyme mono-amine oxidase

In vitro irradiation of the rabbit platelets was realized by centrifuging 4 5 ml

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ORIGIN OF RADIATION RELEASED SEROTONIN IN RABBITS AND MICE

T S VENINGA, J KERKSTRA and J WAGENAAR

It is generally agreed at present that the early rise of plasma and urinary serotonin (5-HT) levels caused by moderate doses of ionizing irradiation are due to a release of 5-HT from the body stores (ALTMAN et coll 1970, STREFFER 1969)

For practical purposes, i e for the localization of injury after radiation accidents more or less specific biochemical indicators are of meaningful value (Bio chem Indicators of Radiation Injury in Man), 5-HT has been mentioned as such It therefore appeared worthwhile to investigate the origin of the 5-HT present in body fluids after exposure to several types of ionizing irradiation 5 HT is bound to subcellular structures in various tissues throughout the body, significant quantities occur in the brain, lungs, liver, intestinal tract, spleen, skin and blood platelets (GARATTINI & VALZELLI 1965) Partial body irradiation has enabled a number of these organs to be examined as being the possible potential sources of the 5-HT released into the blood plasma, the experiments were mainly executed with moderate doses of roentgen irradiation, a few were carried out with 14 MeV neutron irradiation All indicated the intestinal tract as the principal site of origin for the enhanced amount of free 5-HT in the circulation

Methods Random bred Chinchilla rabbits of about 3 kg and institute inbred grey mice weighing between 20 and 30 g were used The rabbits were individually

Submitted for publication 19 December 1972

ixed to an operating table after being anaesthetized with 25 mg/kg sodium pentobarbital Both animals species were kept without food for 24 hours prior to the experiments

Partial body irradiation was performed by covering the non irradiated parts of the body with 4 mm lead and employing a Philips Muller MG 300 apparatus at 200 kV, 15 mA, and 0 5 mm Cu and 0 5 mm Al filtration The dose rate varied with the focus to object distance In rabbits the average dose rate as measured in cadavers by a Philips Universal dosimeter was 45 rad/min and in mice amounted to 74 rad/min for all regions irradiated To obtain platelet irradiation both carotid arteries of the rabbits were bared for 3 cm, a piece of 4 mm lead measuring 5 cm × 3 cm was then placed underneath and the whole animal covered by 4 mm lead with a 3 cm × 3 cm hole above the carotids The dose rate measured was 700 rad/min, a value which was obtained with the 0 5 mm Al filter only

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V_e = volume of the exposed carotid part in ml
V = total blood volume in ml
t = exposure time in s
D_s = radiation dose per s

Venous blood in 4 5 ml samples from the ear for the first three samples and from one of the carotid arteries for the next three samples were collected in plastic tubes containing 0 5 ml EDTA 5 % The first two samples were taken prior to irradiation Blood sampling from the mouse was executed by orbita extraction 0 8 ml blood being collected into 0 2 ml EDTA 2 5 % in physiologic saline All samples were kept at 4° C and spun at 1 250 g in a cooled centrifuge for 10 min Plasma was withdrawn by aspiration and respun at 1 250 g for 30 min Two samples of the platelet free murine plasma were pooled All samples were analysed for their 5 HT content by the method of ASHCROFT et coll (1963) Hemolytic samples were discarded To prevent breakdown of the blood 5 HT the animals were intraperitoneally injected 30 min before the start of the experiment with either 10 mg/kg tranylcypromine or 10 mg/kg iproniazide, both are well known inhibitors of the enzyme mono-amine oxidase

In vitro irradiation of the rabbit platelets was realized by centrifuging 4 5 ml

Table 1

The effect of 250 rad roentgen to various regions on the blood plasma level of 5-HT in the rabbit n represents the number of plasma samples

Irradiated region	5 HT ng/ml plasma (Mean ± SE)				
	Control		5 min		
		n		n	p
Total body	100 ± 00	16	140 ± 10	8	<0 001
Head	160 ± 10	22	160 ± 10	11	n s
Thoraco gastric region	210 ± 10	27	220 ± 20	11	n s
Abdomen	160 ± 10	21	230 ± 10	8	< 0 001
Abdomen sham irradiated	120 ± 10	8	140 ± 20	4	n s
Abdomen splenectomized	60 ± 10	18	110 ± 10	9	< 0 0025

samples of whole blood replenished with 0 5 ml EDTA 5 % at 600 g cold for 15 min Platelet rich plasma was obtained by suction and dispersed in a 5 cm plastic Petri dish Non-irradiated and sham-irradiated samples of the same animal served as controls The samples were centrifuged at 1 250 g at various intervals after irradiation and treated as described above for the 5-HT estimation

The areas of the rabbit irradiated were the whole body, the head, the thoraco-gastric region, including the liver, and the abdomen intact and 72 hours after splenectomy Abdominal sham-irradiation was also performed The irradiation pattern for mice was virtually similar, but the irradiation of blood platelets and of the thoraco-gastric region was omitted The number of animals in each group varied, it can be deduced from the n value in the tables

For the neutron exposure the mice were placed in 2 5 cm perspex tubes with a wall thickness of 0 2 cm, five of the tubes being grouped vertically around the 14 MeV neutron source at an ax to ax distance of 4 5 cm to the ion tube of the generator A dose of 600 rad was delivered to the central part of the abdomen which was perpendicularly positioned in relation to the neutrons emitted The dose was calculated from flux measurements with a value of 6 7 $\times 10^{9}$ rad/n $\times$ cm $^{-2}$

Results

Rabbits and mice bore a remarkable resemblance in plasma 5-HT response after irradiation of the various body regions Only after irradiation of the abdomen was a significant increase in the plasma 5-HT observed (Tables 1, 2, 3,

Table 1 (*cont*)

15 min			30 min			60 min		
	n	p		n	p		n	p
110±10	7	n s	110±10	8	n.s	100±00	8	n s
180+20	10	n s	180+10	11	n s	180+10	11	n.s
240±20	13	n s	220±20	13	n s	200+20	13	n s
210±20	8	0 01	200+10	10	< 0 05	190+20	10	n s
130+20	4	n s	130±20	4	n s			
80 10	9	n s	80±20	9	n s	100+20	8	n s

4) This increase was already present immediately after the irradiation treatment The response in rabbits levelled off in the course of 1 hour (Figure) and reached normal values at 4 hours Exposure of mice to 14 MeV neutrons also led to an increase in the plasma 5 HT (Table 5), comparable to that after roentgen irradiation of whole mice Tables 3 4)

Splenectomy failed to prevent the response to irradiation of the abdomen The control values for the plasma 5 HT lay at the level of approximately a factor 2 1/2 times lower than normal in the rabbits Abdominal irradiation of normal mice caused 5 HT values significantly less than those appearing after total body exposure as well as those in abdominally irradiated splenectomized mice (Tables 3 4)

Irradiation of rabbit platelets in vitro with roentgen doses up to 10 000 R was ineffective (Table 6)

Discussion

Early radiation induced changes in the 5 HT content of internal organs generally correlate with the changes observed in the excretion pattern of the amine after irradiation Early observed decreases in the intestinal tract thus correspond with increases in the blood and urine of 5 HT as well as of its metabolite 5 hydroxyindoleacetic acid (5 HIAA) (Table 7) Organs other than the intestinal tract in general do not react Irradiation of only the abdomen results in a significant increase in the plasma 5 HT level in rabbits as well as in mice Since splenectomy fails to influence this effect there appears to be justification for indicating the intestinal tract as the source of the released 5 HT

Table 1

The effect of 250 rad roentgen to various regions on the blood plasma level of 5 HT in the rabbit n represents the number of plasma samples

Irradiated region	5 HT ng/ml plasma (Mean ± SE)				
	Control		5 min		
		n		n	p
Total body	100±00	16	140±10	8	<0 001
Head	160±10	22	160±10	11	n s
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Abdomen	160±10	21	230±10	8	< 0 001
Abdomen sham irradiated	120±10	8	140±20	4	n s
Abdomen splenectomized	60±10	18	110±10	9	< 0 0025

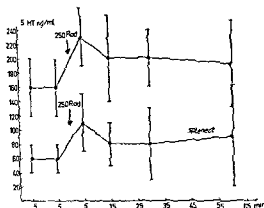
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Results

Rabbits and mice bore a remarkable resemblance in plasma 5 HT response after irradiation of the various body regions Only after irradiation of the abdomen was a significant increase in the plasma 5-HT observed (Tables 1, 2, 3,



5-HT concentrations of rabbit blood plasma at different intervals before and after irradiation with 250 rad roentgen to the abdominal region. The upper curve gives the data of normal animals, the lower curve is constituted of data obtained after splenectomy

It is reasonable to assume that the 5 HT released will enter the blood stream. A part will become oxydized by the enzyme mono-amine oxidase (if this is not inhibited) to 5-HIAA. This process will lead to an increase in the urinary 5-HIAA (Table 7), another part of 5-HT, released by the irradiation, can be excreted as such. An increase in urinary 5-HT has been observed in some animal species (BRINKMAN & VENINGA 1962).

Splenectomy in the rabbits leads to an apparent decrease in the original 5 HT level in blood plasma. This means that in this animal species, in contrast to the mouse, the spleen significantly contributes to the normal level of the plasma 5 HT. The spleen in the mouse has been demonstrated to be involved in a 5 HT retentive process (RITZEN et coll 1965). Hence, free circulating labelled 5-HT was rapidly taken up by the spleen, and a number of other organs such as the lungs, bone marrow, adrenal medulla and the thyroid. This uptake might explain the difference in the rise of plasma 5-HT after abdominal irradiation of normal

Table 4

Comparison of some regions of mice irradiated with either roentgen or 14 MeV neutrons with regard to the release of 5-HT into the blood plasma

Regions compared	Respective 5 HT values Mean ± SE		Significance (p)
Total body Abdomen	80 ± 4 2	60 ± 2 2	< 0 001
Abdomen Abdomen splenectomized	60 ± 2 2	70 ± 3 4	0 001
Total body roentgen/Total body neutrons	80 ± 4 2	95 ± 6 0	n.s

Table 2

5-HT concentrations in rabbit blood plasma after in vivo roentgen irradiation of thrombocytes during MAO inhibition

Roentgen dose (rad)	5-HT ng/ml plasma			
	Control		Experimental	
			5 min	30 min
60	190	200	120	110
60	—	—	230	220
90	210	210	170	150
90	190	250	250	180
90	170	140	130	190
90	180	170	130	130
170	130	120	120	120
200	—	—	110	110
250	180	180	170	170
Average ± SE	180 ± 9		160 ± 17	150 ± 13

The intestinal mucosa contains the bulk of body 5-HT in its enterochromaffin cells (GARATTINI & VALZELLI 1965). The amine is stored in granules known to be sensitive to roentgen doses of 500 to 1 200 R as observed during the first hours after treatment (UHER 1958, ANSARI et coll. 1962). At a later stage, i.e. 12 hours post irradiation, the granules present a normal quantitative and qualitative appearance (ANSARI et coll. 1962, PENTTILLA & KORMANO 1971). The present observations are in accordance with these findings as well as with those that demonstrated a drop in the intestinal 5-HT content early after irradiation (WILLOUGHBY 1960, PENTTILLA & KORMANO 1971).

Table 3

The effect of 575 rad roentgen to various regions on the blood plasma level of 5-HT in the mouse. C — control, E — experimental. The experimental samples were collected immediately after irradiation

Irradiated region	5-HT ng/ml plasma Mean ± SE		Number of samples		Significance (p)
	C	E	C	E	
Total body	50 ± 2.8	80 ± 4.2	13	25	0.001
Head	50 ± 2.8	50 ± 2.9	13	12	n.s.
Head + Thorax	60 ± 2.9	50 ± 2.7	12	14	n.s.
Abdomen	50 ± 2.0	60 ± 2.2	25	55	0.005
Abdomen splenectomized	50 ± 3.3	70 ± 3.4	9	19	0.002

Table 7

The effect of roentgen irradiation on the 5-HT content of different organs as well as on the concentration of 5-HIAA in urine as reported by several authors The upper part of the table indicates values obtained after shorter intervals after roentgen exposure, whereas the middle part presents longer term values

Organ	Animal	Dose (R)	Effect	Interval (hours days)	Authors
5 Hydroxytryptamine					
Liver	Mouse	810	none	0—2h	MELCHING et coll (1960)
Spleen	Mouse	810	none	0—2h	MELCHING et coll (1960)
Stomach	Rat	1 025	drop	3h—15d	EISEN et coll (1956)
Intestine	Rabbit	500—2 000	drop*	0—3h	MATSUOKA et coll (1966)
Intestine	Rat	1 500	drop	24h	WILLOUGHBY (1960)
Intestine	Mouse	1 000—3 000	drop	12—24h	PENTTILÄ & KORMANO (1971)
Brain	Rat/guinea pig	500—1 000	rise	0h	CHERNOV & RAUSCHENBACH (1960)
Brain	Rat/mouse	810	none	0—2h	MELCHING et coll (1960)
Brain	Rat	900—4 000	none	0—24h	RANDIĆ et coll (1961)
Hypothalmus	Rat	1 000	drop	2h	RENSON & FISCHER (1959)
Blood	Rat	800	rise	6h	FRANZEN et coll (1963)
Blood	Mouse	250 600	none	0—5d	MOMMA et coll (1967)
Urine	Rat	400—800	rise	3h—21d	FRANZEN et coll (1963)
Skin	Rat	600—1 000	drop	5—10d	EISEN et coll (1956)
Skin	Rat	600	drop	6d	LEITCH et coll (1957)
Spleen	Rat	450—900	drop	1—6d	ERSHOF et coll (1962)
Spleen	Rat	600	rise→drop	2—4d, 9d	LEITCH et coll (1957)
Intestine	Rat	450—900	drop	1—6d	ERSHOF et coll (1962)
Intestine	Rat	600	rise→drop	6d, 6—16d	LEITCH et coll (1957)
Intestine	Mouse	1 000 3 000	none, drop	2—4d	PENTILLA & KORMANO (1971)
Brain	Rat	900			
Blood	Rat	900	none	6d	ERSHOF & GAL (1961)
Blood	Rat	600	drop	6d	ERSHOF & GAL (1961)
			drop	4 9d	LEITCH et coll (1957)
5 Hydroxyindoleacetic acid					
Urine	Rabbit	1 000	rise	?h	FISCHER & RENSON (1959)
Urine	Rat	810	rise	0—2h	MELCHING et coll (1960)
Urine	Rat	800	rise	6—24h	RANDIĆ & SUPEK (1962)
Urine	Rat	200—800	rise	6—12h	DEANOVIĆ et coll (1963)
Urine	Frog	400	rise	4h	BRINKMAN & VENINGA (1962)
Urine	Man	200—300	none	24h	BETTENDORF et coll (1959)
Urine	Man	150 rad	rise	24h	SMITH & LANGLANDS (1966)

* As reflected in depression of intestinal motility

Table 5

The effect of 14 MeV neutron irradiation directed to the abdomen of mice upon the blood plasma level of 5-HT

	5 HT ng/ml plasma		
	Controls	5 min post—600 rad 14 MeV neutrons	
	50	120	120
	90	110	80
	50	40	90
	50	80	90
	80	90	90
	60	70	140
	60	40	140
	90	130	110
	60	70	120
		110	110
		50	110
		80	
Av ±SE	65±5 3 (n = 9)	95±6 0 (n = 23)	

Difference 30±1 4
p = 0 002

Table 6

5 HT in rabbit plasma after in vitro irradiation of whole blood

Roentgen dose (R)	5 HT ng/ml plasma			
	Control		Experimental	
			5 min	15 30 min
1 000	90	90	—	110
2 000	100	100	100	110
5 000	100	100	110	110
10 000	60	90		60
10 000	140	150	140	170
	180			
Average ±SE	110±10		120±15	110±18

able site from whence the 5 HT was originating Further investigation of the liberation as a potential indicator of radiation injury in the abdominal region would appear to be required

ZUSAMMENFASSUNG

Der Blutplasmaspiegel von freiem 5 Hydroxytryptamin (Serotin 5 HT) wurde bei Kaninchen und Mausen im Anschluss an Partialkorper Rontgenbestrahlung um die Quelle der 5 HT Freisetzung festzustellen bestimmt Die Ergebnisse deuten darauf hin dass der Intestinaltrakt die Stelle ist von der am wahrscheinlichsten 5 HT herstammt Weitere Untersuchungen erscheinen notwendig um die Freisetzung als potentiellen Indikator eines Strahlenschadens des abdominalen Gebietes verwenden zu konnen

RÉSUMÉ

Les auteurs ont determine le taux dans le plasma sanguin de la 5 hydroxytryptamine libre (serotonine 5 HT) sur des lapins et des souris apres une irradiation regionale par les rayons roentgen pour identifier la source de la 5 HT liberee Les resultats indiquent que l intestin est la source la plus probable de la 5 HT Il est necessaire de continuer les recherches sur la liberation de la 5 HT comme indicateur possible de radio lesion dans la region abdominale

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The response of 5-HT observed was not restricted to roentgen irradiation, 14 MeV neutron irradiation also proved to be effective if mainly directed to the abdomen of mice The increase in plasma 5-HT observed was even somewhat more marked although the difference was not significant if compared to the total body roentgen irradiated mice

The reaction on different types of irradiation might represent an early biochemical indicator of radiation injury to the abdominal region It might then be preferable to class the reaction as dose dependent Although a linear relationship between radiation dose and 5-HIAA excretion in the urine of rats has been established (DEANOVIC et coll 1963), a tentative trial in rabbits failed to indicate a dose relationship for the roentgen induced 5-HT increase in the blood plasma (VENINGA, unpublished)

Another uncertainty concerns the 5-HT response in man SMITH & LANGLANDS (1966) observed a rise in the urinary 5-HIAA content of patients receiving partial body irradiation with 150 rad However, BETTENDORF et coll (1959) were unable to demonstrate significant differences in 5-HIAA excretion in patients treated with roentgen irradiation A complication is produced by the alimentary 5-HT intake in human subjects giving rise to fluctuations in the normal excretion pattern of 5-HIAA This may turn out to be a significantly counteracting factor in the development of 5-HT as a biochemical indicator of local radiation injury For the present however it nevertheless seems worthwhile to extend investigations to other mammalian species so as to obtain an impression of the generality of the phenomenon

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Blood plasma levels of free 5 hydroxytryptamine (serotonin 5 HT) have been determined in rabbits and mice following zonal roentgen irradiation of the animals to identify the source of the 5 HT released The results indicated the intestinal tract as the most prob

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N0	N1	N2	N3	Total
12	2	0	0	14
21	1	0	1	23
34	4	1	5	44
4	3	1	7	15
71	10	2	13	96

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Table 3

Treatment of primary tumour

	Total	Curative	Palliative
	32	24	8
	33	32	1
n	3	1	2
	27	27	0
	1	–	–
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ACTA RADIOLOGICA

OFFICIAL ORGAN OF THE RADIOLOGICAL SOCIETIES OF
DENMARK FINLAND NORWAY AND SWEDEN

Vol 12 THERAPY PHYSICS BIOLOGY 1973
Fasc 6 December

CARCINOMA LINGUAE

A series of 96 patients

O ELBROND, A P ANDERSEN and K JORGENSEN

A total of 96 patients with histologically confirmed carcinoma of the anterior two thirds of the tongue was treated during the period of 1946—1965 Those with involvement of the posterior third of the tongue have not been included as this region by the rules of the UICC, belongs to the oropharynx The series is distinguished by regular controls of all the patients after their primary treatment Histology revealed epidermoid carcinoma in 92 patients, adenocarcinoma in 2 and malignant papilloma in 2 patients

The age and sex distributions in the material appear in Fig 1 The age distribution is in accordance with most other series with a maximum age of 60 to 80 years The latter distribution is however remarkable because females predominated (54/40) In most other series (MARTIN et coll 1940, FRAZELL & LUCAS 1962 males formed a considerable majority However during the last years reports have indicated that the number of males with carcinoma of the tongue is diminishing (CONLEY 1967, SPIRO & STRONG 1971)

From the Radium Centre (Directors Sigvard Kaae and A P Andersen) and the E N T Department (Directors H C Andersen O Elbrond and O Jepsen) Aarhus University 8000 Aarhus Denmark Supported by a grant from the Danish Anti Cancer League Submitted for publication 16 April 1973

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T3	34	4	1	5	44
T4	4	3	1	7	15
Total	71	10	2	13	96

The primary symptom was a lump or ulceration with a history in 75 per cent of patients of less than 6 months (72/96) and of 6 to 12 months in 8 per cent (8/96) The duration of the symptoms was more than a year in 6 per cent of patients, no information was available on this point in 4 patients

All the patients are classified retrospectively by the TNM system in accordance with the rules of UICC (Table 1) from detailed descriptions, drawings and photographs 'Deep infiltration' (T3) is defined as infiltration of more than 5 mm

The primary tumour at the time of the histology was, in more than half the patients, in an advanced stage, i e T3 or T4 (Table 2) In addition 25 patients (26 per cent) at this time had regional lymph node metastases and one patient had distant metastases This TNM distribution is the same as in other series (Horiuchi & Adachi 1971) The tumour was on the lateral borders of the tongue in 71 per cent of the patients, on the under surface in 16 per cent, on the dorsum in 8 per cent and on the apex in 1 per cent of the patients, the entire tongue was invaded in 4 per cent There was no difinite lateralisation

Table 3

Treatment of primary tumour

	Total	Curative	Palliative
External irradiation	32	24	8
Implantation	33	32	1
External irradiation + implantation	3	1	2
Surgery	27	27	0
No treatment	1	—	—
Total	96	84	11

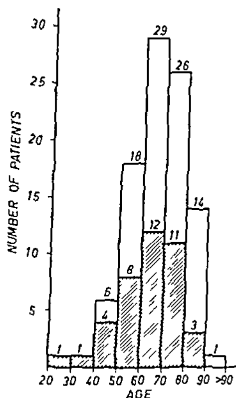


Fig 1 Distribution by age and sex ▨ 40 males ☐ 56 females

Table I

TNM classification of malignant tumours of the tongue (UICC 1968)

T	*Primary tumour (tongue)*
T0	No evidence of primary tumour
T1	Tumour measuring 2 cm or less in its largest dimension strictly superficial or exophytic
T2	Tumour measuring 2 cm or less in its largest dimension with minimal infiltration in depth
T3	Tumour measuring more than 2 cm in its largest dimension or tumour with deep infiltration irrespective of size
T4	Tumour producing complete fixation of the tongue or extending to more than one neighbouring region
N	*Regional lymph nodes*
N0	No palpable nodes
N1	Movable homolateral nodes
N2	Movable contralateral or bilateral nodes
N3	Fixed nodes
M	*Distant metastases*
M0	No evidence of distant metastases
M1	Distant metastases present

Table 7

Regional lymph node metastases Primary metastases indicate metastases present at the time of the diagnosis of the primary tumour Secondary metastases indicate metastases occurring after the initial treatment of the primary tumour

Total number of cases	Number of primary metastases	Number of secondary metastases	Total number of metastases	No metastases
96	25 (26 %)	35 (36 %)	60 (62 %)	36 (38 %)

plantation Twenty seven patients had a primary operation consisting of partial glossectomy and one patient with bilateral regional lymph node metastases and distant metastases received no treatment

Only in 84 patients was the intention of treatment curative Table 4 presents the *results of treatment* by external irradiation with a curative intention Only in 6 patients was it possible to control the primary tumour, but it has to be pointed out that 20 of these were T3 and T4 cases The results of treatment by implantation with a curative intention appear in Table 5 The primary tumour was controlled in 18 patients but it must be observed that only in 9 out of 19 T3 cases did this succeed *Table 6 presents the results of surgical treatment* (partial glossectomy) of the primary tumour This was controlled in 16 patients mostly with T1 or T2 conditions (19/27)

The results of those treated by three different forms of treatment are not comparable, inter alia because the TNM classification is not the same It must be noted however in general that *the recurrency rate is high*, particularly in the T3 and T4 groups However, it was possible in a number of instances to control the local tumour by supplementary treatment, most often by surgery In all, the primary tumour had to be controlled in 58 patients (60 per cent)

The prognosis in carcinoma of the tongue is also determined by the incidence of regional lymph node metastases and the possibility *of their effective* treatment The incidence of regional metastases, primary as well as secondary, appears in Table 7 The number, especially of *the secondary* is considerable

Most metastases occurred in the mandibular and the upper jugular lymph nodes Possible bilateral or contralateral nodes were evident in 14 (23 per cent) of patients 8 cases were primary and 6 were secondary metastases

As already mentioned, the 96 cases of carcinoma of the tongue have been grouped according to the TNM classification The *secondary* metastases were divided following the same principles and related to the T classification of the primary tumours (Table 8) Eleven cases of distant metastases occurred in the period of observation

Table 4

Primary tumour. Curative external irradiation

	No	No recurrence	Recurrence
T1	2	2	0
T2	2	1	1
T3	12	3	9
T4	8	0	8
Total	24	6	18

Table 5

Primary tumour. Curative implantation

	No	No recurrence	Recurrence
T1	3	3	0
T2	10	6	4
T3	19	9	10
T4	0	0	0
Total	32	18	14

Table 6

Primary tumour. Curative surgery

	No	No recurrence	Recurrence
T1	9	6	3
T2	10	6	4
T3	7	4	3
T4	1	0	1
Total	27	16	11

The principles of treatment have changed during the 20-year period. Only during the last 3 years of this period has ^{60}Co high voltage therapy been available and in the rest of the period conventional irradiation was used.

The principles of treatment are presented in Table 3. Thirty-two patients received primary external irradiation, and in 2 patients this was given as peroral irradiation. The treatment in 33 patients consisted of implantation with gold seeds, while 3 patients received a combination of external irradiation and im-

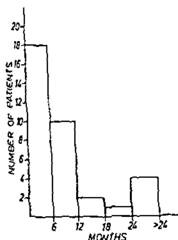


Fig 4 Secondary metastases Time of diagnosis

in the group with metastases related to that in the entire series (Fig 3, revealed no definite bias This is also valid if the groups of males and females are analysed separately

Fig 4 gives by far the largest number of secondary metastases occurring within the first year after the primary treatment (28/35), during this period the patients should be seen at brief intervals Nineteen of the 35 secondary metastases were fixed at the time of observation even though the patients were controlled every second month 13 of the primary metastases were also fixed All such patients 19 + 13) with fixation of the lymph nodes died All the patients with contralateral or bilateral metastases also died even though the metastases were movable

Table 9

Incidence of secondary lymph node metastases

	No of patients	No of secondary regional lymph node metastases
N recurrence of pr mary tumour	35	14 (40 %)
Recurrence f primary tumour or residues	36	21 (58 %)
Total	71	35 (49 %

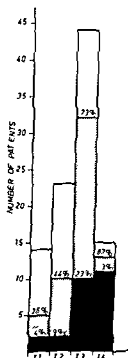


Fig 2

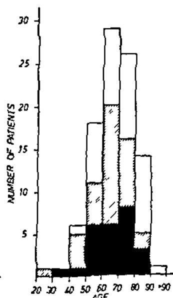


Fig 3

Fig 2 T classification ☐ no metastases ■ primary metastases ▨ secondary metastases

Fig 3 Distribution by age (For symbols see Fig 2)

The cases of metastases as well as the distribution of the entire series by the T classification are evident from Fig 2, this is also conveyed in Tables 2 and 8, but is more apparent in the diagram As excepted the rate of metastases is highest in the T3 and T4 groups The 60 cases of regional lymph node metastases may however be considered from other aspects The age distribution

Table 8

Secondary lymph node metastases

	Movable homolateral nodes	*Movable contralateral or bilateral nodes*	*Fixed nodes*	*Total*
T1	3	0	0	3
T2	4	1	3	8
T3	6	2	14	22
T4	0	0	2	2
Total	13	3	19	35

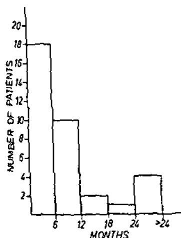


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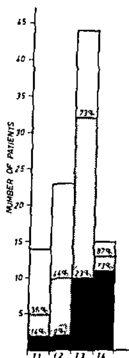


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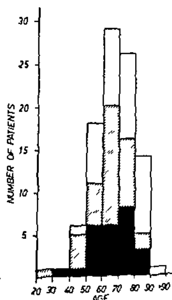


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T2	4	1	3	8
T3	6	2	14	22
T4	0	0	2	2
Total	13	3	19	35

employed only in the latter part of this period in 5 cases of residual tumour or recurrence in all of which it failed to control the disease

Fig 5 presents the survival curve for the entire series the 5 year survival rate being 30 per cent (and corrected for mortality from other causes 39 per cent) The survival curves for each of the T groups appear in Fig 6 The difference between the T1 and T2 groups on one hand and the T3 and T4 groups on the other is important especially when it is rememered that most of the patients were in the T3 and T4 groups

Discussion

The results of treatment of carcinoma of the tongue remain still far from satisfactory as evident from the present series This refers both to the primary tumour and the regional lymph node metastases Many circumstances contribute to the disappointing results and have to be considered when the results are evaluated and prospective planning is carried out Before the principles of treatment are discussed however certain facts on the aetiology and disposition may be helpful in assessing any possible prophylaxis

The basic aetiology of carcinoma of the tongue is not known but many factors seem to dispose to the development of malignancy Leukoplakias of certain types are considered as premalignant affections (CAWSON 1969 PINDBORG et coll 1968) The abuse of alcohol and tobacco is by several authors (EGGERS 1934 FRASER 1932 JACOBSSON 1948 TRIEGER et coll 1958) stated as a disposing condition perhaps preceded by leukoplakias Luetic leukoplakias on the tongue display a tendency to become malignant (MARTIN et coll 1940), even though the infection is treated and the Wassermann is negative (CAWSON 1969)

Patients with the Plummer Vinson syndrome are inclined to develop carcinoma of the oral cavity as well as of the postcricoid region (AHLBOM 1933 WYNDER & FREYER 1958) Effective treatment of this condition may for this reason be of importance CAWSON (1969) is of the opinion that chronic irritation from defective teeth and poor hygiene of the mouth are not carcinogenic

Effective control of the local tumour with an extension of 4 cm or less may be obtained by partial glossectomy in more than 80 per cent of patients (SPIRO & STRONG 1971) and by implantation of ^{192}Ir in as many as 95 per cent of patients (PIERQUIN et coll 1971) Implantation as the primary treatment is indicated STEENSMA 1972) to give no or only a moderate loss of tissue substance a recurrence after this may frequently be controlled by radical surgery Primary surgical extirpation (partial glossectomy) carries a loss of tissue sub

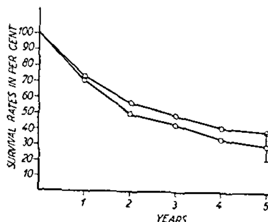


Fig 5 Carcinoma of the tongue 96 patients Survival curves (upper corrected lower uncorrected)

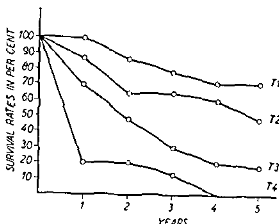


Fig 6 Carcinoma of the tongue 96 patients Survival curves for each of the 4 groups T1 71 % T2 48 % T3 18 %, T4 0 %

Another fact of importance for the incidence of metastases is the degree of success of the initial treatment of the primary tumour Table 9 indicates that the majority of the secondary metastases occurred in the group of patients with local recurrences or malignant residues (58 against 40 per cent)

Thirty-four patients with regional lymph node metastases were treated by surgery (radical neck dissections—in 3 patients on both sides) and 22 by radiation therapy, 4 patients received no treatment (Table 10) The treatment results of the regional lymph node metastases after surgery were disappointing It must however be borne in mind that these results reflect the treatment policy over a period of twenty years *Composite resections* (neck dissections *in* continuity with resection of the primary tumour, residues or recurrence) have been

Table 10

Treatment of regional lymph node metastases

	No	Died from condition	Died from other causes	Alive after 5 years without *recurrence*	Alive after 5 years with *recurrence*	Died from condition after 5 years
Neck dissection	34	25	0	6	3	3
				9 (26 %)		
Radiation	22	18	2	0	2	1
				2 (9 %)		
No treatment	4	4	0	0	0	0
Total	60	47	2	11 (18 %)		4

operation that attempts sometimes to preserve the continuity of the surgical specimen without sacrifice of the mandible may be performed

Whether prophylactic irradiation of the submandibular and upper jugular groups of lymph nodes on both sides of the neck can ever reduce the incidence of secondary lymph node metastases is still not clear Reports in the literature (HORIUCHI & ADACHI 1971) indicate however that this may occur, a controlled trial would be of help Again it is not evident whether preoperative high voltage irradiation will improve the prognosis in N1 and N2 and especially N3 cases

The prognosis in carcinoma of the tongue is still, especially in the more advanced cases, constituting the majority, most serious Improvement of the prognosis seems to be a possibility in view of more active radiation therapy and surgical procedures

Conclusions

The series comprised 96 patients with histologically confirmed carcinoma of the tongue primarily treated by external irradiation, implantation with gold seeds a combination of external irradiation and implantation and partial glossectomy One patient received no treatment The incidence of recurrence was in general high especially in the T3 and T4 groups In all it was possible to control the primary tumour in 58 patients (60 per cent)

Twenty five patients (26 per cent) had primarily metastases in regional lymph nodes and 35 patients (36 per cent) developed metastases secondarily More than half (32/60) of the regional lymph node metastases were fixed All these patients died of the condition Radiation therapy of the nodal metastases (22 patients failed to control the disease

Only 6 out of 34 patients with radical neck dissections survived 5 years without evidence of recurrence The 5 year survival for the entire series was 30 per cent and after correction for mortality from causes other than cancer, 39 per cent The analysis of the present series of cases of carcinoma of the tongue and reports in the literature indicate that a much more active treatment policy, usually as preoperative high voltage irradiation and radical surgical procedures combined must be applied if the prognosis is to be improved

SUMMARY

A material of 96 patients with carcinoma of the tongue is reviewed and analysed This had been treated with external irradiation implantation with gold seeds and a combination of both as well as by surgery The total mortality reached 39 per cent It is suggested that an improvement in the results might be obtained by a more active policy of treatment

stance, which with a recurrence will impede further radical surgical procedures. Partial glossectomy and treatment by implantation have been followed by almost identical results in the T1, T2 and T3 groups of the present series. The frequency of recurrences is high, especially in the T3 groups, and rises to more than 50 per cent.

FRAZELL & LUCAS (1962) have indicated that in 30 per cent of advanced cases (the primary tumour with more than 2 cm spread with infiltration and perhaps regional lymph node metastases) it is possible to control the condition by radical resections en bloc. (This implies neck dissection with removal of the primary lesion together with intervening structures.) CONLEY (1967) and WARD et coll. (1959) considered combined resections of this type to give the best results.

Other authors such as MONTANA et coll. (1969), HORIUCHI & ADACHI (1971) indicated that a combination of external and interstitial irradiation or external irradiation alone, supplemented by radical neck dissections in case of regional lymph node metastases, produce results equal to those followed by radical combined resections. Preoperative external irradiation (4 000 rad/3 weeks) followed by radical resection was suggested by JAKOBSSON & WERSALL (1973) to produce promising results. In only 3 of 20 T3 and T4 cases has it been possible to control the primary tumour by external irradiation (given as a curative intention).

As indicated, the primary and especially the secondary incidence of regional lymph node metastases in carcinoma of the tongue is high. The treatment of the regional lymph node metastases is for this reason of decisive importance to the final result. Treatment of the regional metastases is of course only rational when the primary tumour is or can be controlled. Radical neck dissections offer the best opportunity to control regional lymph node metastases (MARTIN et coll. 1951). It is rarely possible to control lymph node metastases by irradiation alone as has been confirmed in the present analysis.

One of the questions most debated among head and neck oncologists is the value of elective neck dissections. These have not been performed in the present series. The same attitude has been taken as MARTIN et coll. (1951) who argued strongly against such dissections. Analysis of the present series of carcinoma of the tongue has revealed among other things that the incidence of regional secondary metastases was high (35/60) and further, that a considerable number of the secondary metastases (19/35) were fixed when observed in spite of frequent controls (about every two months). These findings now suggest, in agreement with STEENSMA (1972), that elective neck dissections may well be performed when the primary tumour is infiltrating. If necessary this will be in continuity with its resection with the intervening structures. The pull through

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ZUSAMMENFASSUNG

Ein Material von 96 Patienten mit einem Karzinom der Zunge wird *zusammenfassend* dargestellt und analysiert Dieses war mit externer Bestrahlung Implantation mit Gold einer Kombination beider Therapieformen wie auch chirurgisch behandelt worden Die *Gesamtmortalität erreichte 39 Prozent* Es erscheint wahrscheinlich dass verbesserte Ergebnisse durch eine mehr aktive Behandlungseinstellung erreicht werden konnen

RÉSUMÉ

Les auteurs ont passe en revue et analyse une serie de 96 malades atteints de cancer de la langue Ces malades ont ete traites par irradiation externe implantation de grain d or et association de ces deux methodes et aussi par intervention chirurgicale La mortalite totale atteignait 39 pour cent Les auteurs pensent qu une tactique therapeutique plus active pourrait ameliorer les resultats

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Table 1

Carcinoma of the uterine cervix Urography (from the literature)

Stage	No of patients	Patients with ureteric obstruction	
		No	Per cent
I	147	10	7
II	267	33	12
III	272	61	22
IV	84	44	52
Total	770	148	19

the prognosis was similar for cauliflower, discoid and crateriform tumours in stages I, III and IV, the outlook might be slightly more favourable for the cauliflower tumour in stage II

The prognostic significance, if any, of the histologic type of the tumour has attracted much attention Several systems for grading the histologic appearances have been devised, great importance being attached to the degree of anaplasia (KJELLGREN 1958) The radiation dose up to a certain optimum as well as its distribution in the pelvis is another important factor correlated with the outlook (KJELLGREN) Radiographic examinations of the kidneys have been carried out to correlate the radiographic findings with the prognosis (BURNS et coll 1960) Ureteric obstruction before therapy will be of grave prognostic significance The frequency of such involvement differs somewhat in different reports (BURNS et coll, POMEROY 1947, RHAMY & STANDER 1962) (Table 1)

Table 2

Carcinoma of the uterine cervix Distribution of international stages

Stage	No	Per cent
I	128	43
II	118	40
III	38	13
IV	13	4
Total	297	100

FROM THE DEPARTMENTS OF GYNECOLOGIC RADIATION THERAPY (DIRECTOR PROF OLLE KJELLGREN), AND DIAGNOSTIC RADIOLOGY (DIRECTOR PROF G F SALTZMAN), UMEÅ UNIVERSITY, 901 85 UMEÅ, SWEDEN

ISOTOPE NEPHROGRAPHY IN CARCINOMA OF THE UTERINE CERVIX AND ITS PROGNOSTIC SIGNIFICANCE

K E KJORSTAD, O KJELLGREN, L JONSSON and P JUNGHAGEN

A variety of factors may be of prognostic importance following irradiation of carcinoma of the uterine cervix, some of which may be of value whereas other apparently important clinical observations have proved to have little or no significance (KJELLGREN 1958) An advanced clinical stage is undoubtedly associated with an unfavourable prognosis It is often difficult however to determine how far the disease has progressed (HEYMAN et coll 1953) and the system of classification into clinical stages is not completely congruent with the pathologic extent of the lesion (CHERRY et coll 1953), the clinical stage in the individual case permits of no definite prognosis A large number of observations have indicated that the prognosis is less favourable in young than in elderly subjects although the opposite has however also been suggested (LINDELL 1952)

A link has been sought between the gross morphology of the condition and the prognosis (TRUELSEN 1949, BORELL 1953) BORELL reported that while

Submitted for publication 26 March 1973

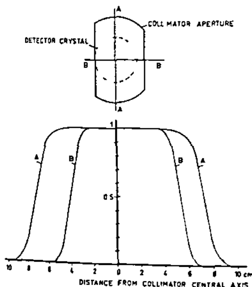


Fig 2 The shape of the collimator aperture and response curve at 6 cm from collimator front

Method The isotope nephrography is carried out by means of 15 μCi ^{131}I Hippuran injected intravenously The recording is generally performed with the patient sitting in a special chair that is adjustable if the patient is to be examined supine (Fig 1) Two scintillation detectors fitted with a shaped collimator are placed over each kidney The collimator, 12 cm long, is able to cover the entire kidney but only a small area of the surrounding tissue and the other kidney The shape of the collimator aperture and the response curve appear in Fig 2 The collimator is asymmetric in the plane across the kidney, it may be rotated and adjusted over the kidney with the steeper part directed towards the other kidney A third detector with a conical collimator to produce a circular field is placed over the heart to record the blood clearance curve and to indicate that the injection is correct (a high initial peak in the curve) The pulses from the detectors are stored in a core memory (Intertechnique's Didac 800 with A 48) working in the multiscaling mode with a time interval of 7 5 seconds The data are presented on a oscilloscope screen and recorded on polaroid film

The initial phase of the isotope nephrogram is characterized by a rapid increase in activity shortly after the injection This is called the vascular spike The second phase discloses a slower increase to a maximum, during which phase the isotope accumulates in the kidney and in the renal pelvis The third phase consists of a more or less rapid fall of activity down to zero as the renal pelvis empties Fig 3 a Experiments have indicated that this pattern is changed in

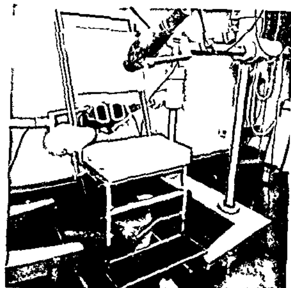


Fig 1 Adjustable chair for isotope nephrography

TAPLIN et coll introduced isotope nephrography in 1956 to provide a simple method of examining renal function, this has found considerable wide clinical acceptance One major problem with this method is the lack of generally accepted standards This applies to the interpretation of the tracings as well as to the conditions under which the examination is performed There may thus be different opinions as to the significance of abnormal findings, but all authors seem to agree that a normal recording eliminates impaired renal function and ureteric obstruction

Only a few papers on isotope nephrography and its clinical application in carcinoma of the uterine cervix have been published VAN VAERENBERGH & DE WINTER (1971) and PATRÍCIO & BAPTISTA (1968) have reported a high percentage of ureteric obstruction even in clinical stages I and II They also considered that the method gives valuable information on the prognosis

The isotope nephrography has been used as a means of following up patients treated for both malignant and benign gynaecologic tumours, and has proved to be a reliable screening procedure, a normal recording excludes urinary tract involvement (RODDICK et coll 1964, KOKKONEN et coll 1971)

Material A total of 297 patients with invasive carcinoma of the uterine cervix were treated during the period 1965 to 1971 This included practically all patients with carcinoma of the uterine cervix in a defined geographic area in the northern part of Sweden with a population of approximately 700 000 All patients underwent urography as well as isotope nephrography before irradiation

The stage distribution in the material appears in Table 2 Forty-three per cent of the patients were allotted to stage I and 40 per cent to stage II

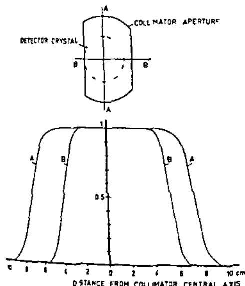


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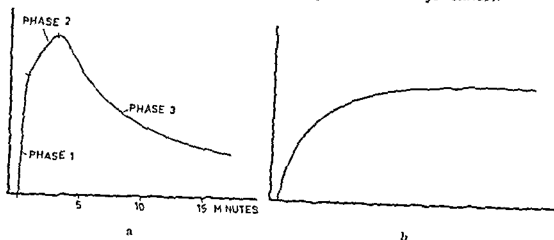


Fig. 3. Isotope renogram. a) Normal. b) Partial ureteric obstruction.

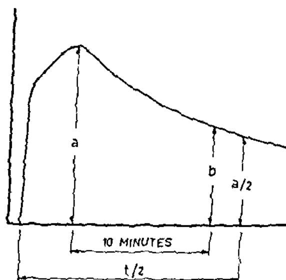


Fig. 4. Borderline between normal and abnormal inclination between the third phase: $a/b > 1.8$ (AURELL) $t/2 < 18$ min (ZUM WINKEL).

ureteric obstruction. Most authors consider the method more sensitive than urography for the detection of ureteric obstruction (Fig. 3 b). Slight ureteric obstruction prolongs the third phase. It is not easy to define the border line between normal and abnormal inclinations of the third phase. ZUM WINKEL (1960) considered half time as the most reliable criterion of obstruction: this is the time in minutes between the injection of the isotope and the point at which the activity has decreased to half the maximum value. The upper limit of the half time, in his opinion, was 18 minutes. AURELL (1971) defines a normal third phase by means of the excretion index: this was the ratio between the maximum activity and the activity measured on the slope of the third phase ten minutes

Table 3

Carcinoma of the uterine cervix Ureteric obstruction (297 cases)

	Abnormal urography No	Abnormal isotope nephrography No
Right	7	16
Left	9	14
Bilateral	11	18
Total	27 = 9 %	48 = 16 %

$\chi^2 = 6\ 7$ d f = 1 0 01 > p > 0 001

after it had reached its maximum He considered an excretion index of 1 8 or more to be normal The Aurell criterion has been employed in the present investigation (Fig 4)

Routine urography was always performed and 40 ml Urografin 60 % injected intravenously The first film was exposed at three minutes without compression followed by films at 13 minutes with compression and further films at 20 minutes with the pressure released This type of urography affords information on the morphology of the urinary tract and only to a lesser extent on the renal function The examination is performed primarily to determine whether ob struction to the ureters exists

Twenty seven or 9 per cent of the 297 urographies were abnormal Signs of ureteric obstruction were present on the right side in 7, on the left side in 9 and on both sides in 11 patients Forty eight of the 297 isotope nephrographies or 16

Table 4

Carcinoma of the uterine cervix Distribution of abnormal findings in different clinical stages

Abnormal urography			Abnormal isotope nephrography	
Stage	No	Per cent	No	Per cent
I	2 128	2	7/128	6
II A	3 59	5	6/59	10
II B	5 59	9	9/59	15
III	11 38	29	17/38	45
IV	6 13	46	9/13	70
Total	27 297	9	48/297	16

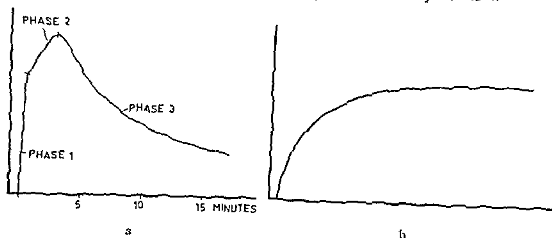


Fig 3 Isotope nephrogram a) Normal b) Partial ureteric obstruction

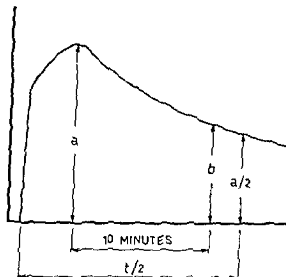


Fig 4 Borderline between normal and abnormal inclination between the third phase $a/b > 1.8$ (AURELL) $t/2 < 18$ min (ZUM WINKEL)

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Table 6

Carcinoma of the uterine cervix Ureteric obstruction and survival after treatment

	Normal isotope nephrography			Abnormal isotope nephrography		
Stage	No	Alive 3 years	Per cent	No	Alive 3 years	Per cent
I	49	42	86	4	2	50
II A	29	21	72	5	2	40
II B	21	12	57	8	2	25
III	16	11	69	9	2	22
IV	2	0	0	7	0	0
Total	117	86	74	33	8	24

χ^2 26 4 d f 1 p 0 001

classified as having negative findings On the other hand, where urography was classified as disclosing ureteric obstruction, the isotope nephrography also revealed obvious signs of obstruction on the same or on both sides A positive urography and a negative isotope examination have never been recorded in the same patient The isotope nephrography in 21 patients disclosed signs of ureteric obstruction whereas the urography was normal

The prognostic significance of abnormal urography and isotope nephrography before treatment appears in Table 5 A total of 232 patients were followed for a year Twenty three of these had an abnormal urography and 41 abnormal isotope nephrography Patients with no signs of ureteric obstruction had an over all survival rate of 92 per cent The survival rate for patients with an abnormal urography was 30 per cent and for patients with an abnormal isotope nephro-

Table 7

Carcinoma of the uterine cervix Ureteric obstruction and survival Positive isotope nephrography and negative urography

Stage	No	Alive 3 years
I	3	2
II A	2	1
II B	5	1
III	3	1
IV	3	0
Total	16	5 (31 %)

Table 5

Carcinoma of the uterine cervix

	Normal urography			Abnormal urography		
Stage	No	Alive 1 year	Per cent	No	Alive 1 year	Per cent
I	98	97	99	2	1	50
II A	43	39	91	3	1	33
II B	43	38	88	4	2	50
III	20	15	75	8	3	38
IV	5	3	60	6	0	0
Total	209	192	92	23	7	30

$\chi^2 = 63.2$, d.f. = 1, $p < 0.001$

	Normal isotope nephrography			Abnormal isotope nephrography		
Stage	No	Alive 1 year	Per cent	No	Alive 1 year	Per cent
I	93	90	97	7	[illegible]	71
II A	41	37	90	5	2	40
II B	39	35	90	8	4	50
III	15	12	80	13	7	54
IV	3	2	67	8	2	25
Total	191	176	92	41	20	49

$\chi^2 = 48.7$ d.f. = 1 p 0.001

per cent were abnormal with signs of ureteric obstruction (Table 3). The isotope examinations thus revealed signs of ureteric obstruction more frequently than the urographies, the frequency of abnormal isotope nephrographies was almost twice as high as that of abnormal urographies. The distribution of abnormal recordings in different clinical stages is given in Table 4.

PATRÍCIO & BAPTISTA (1968) and VAN VAERENBERGH & DE WINTER (1971) came to the conclusion that ureteric obstruction is more frequent on the right than on the left side. This was not confirmed in the present material.

The possibility of false positive isotope nephrographies must be kept in mind. Many factors influence the shape of the curve and several authors have pointed out that a state of dehydration may produce recordings simulating bilateral ureteric obstruction. However, the distribution of abnormal tracings differs little from that evident in urography. Furthermore nearly all patients with abnormal tracing have had multiple examinations and all with possible artefacts have been

ZUSAMMENFASSUNG

Eine Urographie und eine Nephrographie mit Isotopen wurde vor der Behandlung von 297 nicht selektierten Patienten mit einem invasiven Karzinom der Cervix uteri vorgenommen. Sechzehn Prozent wiesen eine abnormale Nephrographie auf, verdächtig auf eine Beteiligung der Ureteren. Diese hatten eine wesentlich ungünstigere Prognose im Vergleich zu den Patienten mit normalen Befunden zur Folge. Patienten mit Zeichen einer Beteiligung der Ureteren, die nur mit der Isotopentechnik und nicht durch die Urographie nachweisbar war, hatten ebenfalls eine schlechte Chance.

RÉSUMÉ

Les auteurs ont fait une urographie et une nephrographie isotopique avant traitement à 297 malades non selectionnees atteintes de cancer envahissant du col de l'uterus. Seize pour cent de ces malades avaient une nephrographie anormale faisant penser à une atteinte urétérale et faisant porter un pronostic beaucoup moins favorable que celles qui avaient des enregistrements normaux. Les malades qui avaient des signes d'atteinte urétérale mis en évidence uniquement par la technique isotopique et non par l'urographie avaient elles aussi un mauvais pronostic.

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graphy 49 per cent This difference between the two groups of patients may have been due to the fact that the latter is a more sensitive method for demonstrating ureteric obstruction than urography performed by the technique used in this material If this be true the prognostic significance of the isotope nephrography will increase as the observation time increases

The three-year survival rate appears in Table 6 A total of 150 patients were followed for three or more years, 117 of these having had normal isotope nephrography Seventy-four per cent or 86 out of these 117 patients survived three or more years Only 24 per cent of the 33 patients with a nephrogram indicating ureteric obstruction lived for three or more years Twenty-one patients had an abnormal isotope examination and a normal urography Sixteen of these patients had a follow-up time of three years or more, the three-year survival rate was 31 per cent (Table 7)

Discussion

Isotope nephrography is easily performed and provides valuable information in carcinoma of the uterine cervix Its chief value lies on the early detection of ureteric involvement, little or no knowledge of the condition of the kidneys and the lower urinary tract is obtained It cannot generally be regarded as a substitute for urography but often affords valuable additional information

Urography delineates the condition of the urinary tracts and demonstrates stasis in the renal pelvis and ureters but slight stasis may be difficult to observe On the other hand isotope nephrography with the above-mentioned criteria for stasis is easy to interpret and seems to be sensitive in revealing even a slight degree of stasis It should be considered as a screening method An abnormal finding calls for further examination with urography A normal isotope tracing practically rules out urography being able to furnish any further information

The fact that patients with abnormal isotope nephrographies have an unfavourable prognosis must indicate that they have more advanced malignancy involving the parametrial tissues

SUMMARY

Urography and isotope nephrography were performed before treatment in 297 unselected patients with invasive carcinoma of the uterine cervix Sixteen per cent had an abnormal nephrography suggestive of ureteric involvement producing a much less favourable prognosis than those with normal recordings Patients with signs of ureteric involvement demonstrable only with the isotope technique and not by urography were also poor risks

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SELACHYL ALCOHOL AS AN ADJUNCT TO RADIATION TREATMENT OF CARCINOMA OF THE CERVIX

O H WARWICK

The alpha glyceryl ethers, or alpha alkoxyglycerols, are widely distributed in nature, occurring in human bone marrow, liver, red blood cells and milk They are most abundant in the liver oil of certain species of shark

Alkoxyglycerols have the formula CH_2OH CHOH CH_2O R with R being a long-chain aliphatic radicle Batyl, chimyl and selachyl alcohols are the most common members of the group The first two have 18 and 16 carbons respectively, in the sidechain and are saturated whereas selachyl, with 18 carbons, is unsaturated

The alkoxyglycerols are absorbed readily from the gut and are of very low toxicity They have anti inflammatory properties (BURFORD & GOWDEY 1968) and are weak inhibitors of tumour growth in experimental animals (ABATUROVA & SHUBINA 1964) They have become best known however for their effect of stimulating red cell, white cell and platelet formation in experimental animals (LINMAN 1960, SANDLER 1949, SUKI & GROLLMAN 1960) and for [illegible]
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Submitted for publication 2 February 1973

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Table 2

Stage of disease

	Group 1		Group 2	
	No	Per cent	No	Per cent
Stage 1	85	42	77	38
Stage 2	86	42	93	47
Stage 3	25	12	29	14
Stage 4	8	4	2	1
Total	204		201	

The investigation was of the double blind type The patients were divided randomly A telephone call was made to a secretary at the London clinic who opened the presenting envelope of a series in which each member had previously been labelled either Group 1 or Group 2

Thus patients were divided into two, hopefully homogeneous groups Each patient was to receive radiation treatment and each patient would, during the course of treatment, take six capsules per day labelled according to the group to which she had been assigned The capsules, indistinguishable in appearance, each contained cottonseed oil as a carrying or blending agent One group however, shown to be Group 1 after breaking the code upon completion of this written report, took capsules which contained, in addition, 100 mg of selachyl alcohol as a natural concentrate, (600 mg per day) Group 2 received capsules which contained only cottonseed oil A dose of 600 mg per day was chosen because this amount corresponded to the highest intake of esters in BROHULT's material

Haemoglobin, white blood cell and platelet values were estimated before treatment and at weekly intervals until radiation treatment was completed

Of the 439 patients, 34 were subsequently excluded, leaving 204 patients in Group 1 and 201 patients in Group 2 The reasons for exclusion are listed below

	Group 1	*Group 2*
Admitted in error	8	7
Insufficient medication	4	5
Errors in medication	2	5
Intent of treatment altered	—	3

Table 1

Distribution of patients by clinic

	Group 1	Group 2
Hamilton	73	69
Kingston	5	4
London	60	66
Ottawa	39	48
Thunder Bay	27	14
Total	204	201

BROHULT (1963) published a report of great practical interest A large group of patients with carcinoma of the cervix was given a mixture of alkoxyglycerol esters during the course of irradiation Compared with a control group, the white cells and platelets were significantly protected from the effects of radiation and the patient survival rates were significantly higher The survival rates, in turn, seemed directly related to the total amount of esters taken

Material and Methods

Because of the clinical importance of BROHULT's observations an investigation was designed to determine whether selachyl alcohol, in conjunction with radiation therapy, might have protective effects on the blood and result in higher survival rates In this respect our investigation differs from that of BROHULT who, for most of her patients, used an ester mixture from Greenland shark liver oil containing alkoxyglycerols with 14—22 C atoms in the side-chain, selachyl alcohol accounting for 60 per cent of the alkoxyglycerol content

Selachyl alcohol was chosen for several reasons It is the predominant alkoxyglycerol in the source material of shark liver oil (BROHULT 1963) and has been reported as more effective than ester mixtures or batyl alcohol in protecting marrow from the effects of radiation (BROHULT 1958) It has also been reported as more effective than batyl alcohol in inhibiting the growth of animal tumours (ABATUROVA & SHUBINA) Moreover, it was readily available in a form easily taken by our patients

Between September 1964 and February 1967, five of the clinics of the Ontario Cancer Treatment and Research Foundation cooperated in the investigation of 439 patients diagnosed as having carcinoma of the cervix Treatment consisted, usually, of two radium insertions followed by external irradiation, the whole being given, where practical, over a four to five week period

Table 5

Differences in blood values before treatment and on completion of treatment

	Category	No	Mean difference (before after)	Standard deviation	t test	Mean difference (Group 1 — Group 2)	t test
Haemoglobin (g %)	Group 1	115	0 58	1 1739	5 2983	−0 1968	−1 1731
	Group 2	108	0 7768	1 3296	6 0716		
White cells	Group 1	120	2 645	2 728 85	10 6178	−114 10	−0 3120
	Group 2	115	2 759	2 874 16	10 2944		
Platelets	Group 1	105	30 380	91 240	3 4119	9 410	0 7563
	Group 2	97	39 790	84 300	4 6489		

In each group only ten patients failed to complete the planned course of irradiation In Group 1 the period of treatment was longer than 5 weeks in 52 cases and in Group 2, in 50 cases

Observations

Of the complications (Table 4) it is difficult to say whether any one may have been related to selachyl alcohol The higher incidence of skin rash in Group 1 may be significant The three cases of jaundice in Group 2 occurred at one clinic early in the investigation and nearly resulted in its termination In retrospect, all cases of jaundice were possibly related to methoxyfluothane anaesthesia Two patients died of coronary and cerebral thrombosis and another of fluid and electrolyte imbalance associated with severe diabetes

One purpose was to determine whether selachyl alcohol had a protective effect on the hemopoietic system This group of patients consisted of those who completed their treatment in five weeks or less and whose haematologic examination had been carried out as planned Table 5 summarizes a comparison of the blood values of the two groups before, and upon the completion of treatment Statistical analysis of the data indicates that during the period of treatment each group had a significant fall in the haemoglobin, white cell and platelet values but that there was no significant difference between the fall noted for each group Thus, no protective effect of selachyl alcohol upon the blood was demonstrated

The five year follow-up of the 405 patients was satisfactory as no patients

Table 3

Radiation treatment

	Group 1	Group 2
Radium only	2	5
External irradiation only	4	7
Radium and external irradiation	198	189
Total	204	201

Patients admitted to the material in error had already been operated upon as part of treatment or were found subsequently to have been incorrectly diagnosed, the correct diagnosis being carcinoma in situ or carcinoma of the endometrium If patients did not take medication for four-fifths of the period of radiation treatment they were excluded as were several who in error received both types of capsule Also excluded were two patients for whom the intent of treatment was altered from radium and external radiation to radium and surgery In another case no treatment was given

The mean age for patients in Groups 1 and 2 was 50 and 52 years, respectively The distribution of patients from the five cooperating clinics is shown in Table 1 Table 2 shows the distribution of stages of the disease within the two groups and Table 3 the type of irradiation

Table 4

Complications

	Group 1	Group 2
Nausea vomiting diarrhoea	10	12
Skin rash	5	1
Infection	2	4
Jaundice	1	3
Thrombosis	2	
Phlebitis	1	—
Haemorrhage	1	
Pulmonary emboli (lipiodol)		1
Pleural effusion	1	
Ruptured ovarian cyst		1
Dehydration		1
Total	23	23

BROHULT's other patients who received medication prophylactically showed a significantly higher survival rate Although not so stated, the reason, presumably, for giving alkoxyglycerols prophylactically was that serum ornithine carbamoyl transferase (S OCT) rises during irradiation (BROHULT 1969) and that the elevation is less when alkoxyglycerols are given prophylactically (BROHULT et coll 1972)

The present report is comparable to BROHULT's of 1963 where alkoxyglycerols were not given prophylactically Confirmation of her more recent work will be awaited with interest by all concerned with the treatment of carcinoma of the cervix

Acknowledgements

This investigation was supported by a grant from the Ontario Cancer Treatment and Research Foundation The author wishes to acknowledge the cooperation of the Directors and staffs of the five participating clinics of the Foundation and the assistance of the Department of Epidemiology and Statistics of the University of Western Ontario in the analysis of data The medication used was supplied by John Labatt Limited, London Canada

SUMMARY

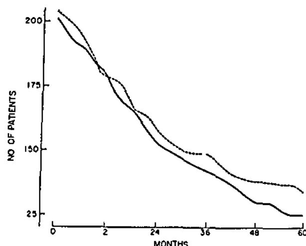
Over 400 patients with carcinoma of the cervix were divided randomly into two groups, one of which received selachyl alcohol 600 mg daily during the course of radiation treatment Analysis of haemoglobin, white cell and platelet values upon completion of treatment and of survival rates over a five year period revealed no significant differences between the two groups

ZUSAMMENFASSUNG

Über 400 Patienten mit einem Cervixkarzinom wurden zufällig in zwei Gruppen aufgeteilt die eine von diesen erhielt täglich im Ver[illegible]
Selachyl Alkohol Die Analyse des Hämoglobins der [illegible]
cyten bis zur Vollendung der Behandlung sowie die [illegible]
Jahres Periode ergaben keine signifikanten Unterschiede zwischen diesen beiden Gruppen

RÉSUMÉ

Plus de 400 malades atteintes de carcinome du col de l'uterus ont ete reparties au hasard en deux groupes dont l'un a eu un traitement quotidien par 600 mg de selachyl alcool au cours du traitement par les radiations L'analyse d[illegible]
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Decline in number of survivors by month, for five years Broken line Group 1 Solid line Group 2

were lost The figure shows, by month, for a five year period, the decline in the number of survivors and reveals no marked difference between the two groups Within Group 1, 70 died and within Group 2, 76

A χ^2-test was carried out of the observed difference in the proportions surviving at the end of five years The results of the test were as follows

$$\chi = 0\ 537,\ 1\ df\ 0\ 50,\ p > 0\ 30$$

Thus, the observed difference fell far short of statistical significance at the 5 per cent level

No significant improvement of survival rates for patients receiving selachyl alcohol during the course of radiation treatment was therefore demonstrated

Discussion

As stated, our investigation began in 1964 with the objective of extending the observations of BROHULT (1963) In 1970, BROHULT et coll reported further experiences on alkoxyglycerols and their use in cancer treatment Over a two year period from 1964 to 1966, a large group of patients irradiated for carcinoma of the cervix also received alkoxyglycerols The form in which the medication was given is not stated About half the patients received medication over an eight day period prior to the irradiation The remainder, as previously, received medication only during the period of radiation therapy and these patients, over the three year period of follow-up, showed no significant difference in mortality from a control group receiving irradiation only Expressed differently, it would seem that BROHULT was unable to confirm the higher survival rates, reported in 1963, for patients receiving alkoxyglycerols during the course of radiation treatment

FROM RADIUMHEMMET (DIRECTOR PROF J EINHORN), THE DEPARTMENT OF ENDOCRINOLOGY AND METABOLISM (DIRECTOR PROF R LUFT), KAROLINSKA SJUKHUSET, AND THE DEPARTMENT OF CLINICAL RADIOPHYSICS (DIRECTOR PROF R WALSTAM), NATIONAL INSTITUTE OF RADIATION PROTECTION, 104 01 STOCKHOLM, SWEDEN

PITUITARY FUNCTION IN LONG-TERM SURVIVAL AFTER RADIATION THERAPY OF NASOPHARYNGEAL TUMOURS

A DE SCHRYVER, J-G LJUNGGREN and I BÄRYD

The effect of external irradiation procedures, as used in the treatment of intracranial malignancy on normal human pituitary function is only incompletely known Both clinical observations after the irradiation of brain tumours (BOUCHARD 1966) and the histology of the pituitary gland following the implantation of radiation sources (NOTTER 1959) indicate that the gland is not particularly sensitive to radiation doses as used in routine external treatment

Most of the observations reported are concerned, however, either with the presence or absence of gross clinical abnormalities such as growth retardation and diabetes insipidus The observation time (3 to 12 and 1 to 13 months, respectively) after irradiation may have been to short for a pituitary lesion to manifest itself with full clinical or laboratory evidence in other investigations (KELLY et coll 1951, PLUNKETT 1957) No regard is paid, of course, to cases in which high concentrated doses are administered, either by proton irradiation (LAWRENCE

Submitted for publication 24 April 1973

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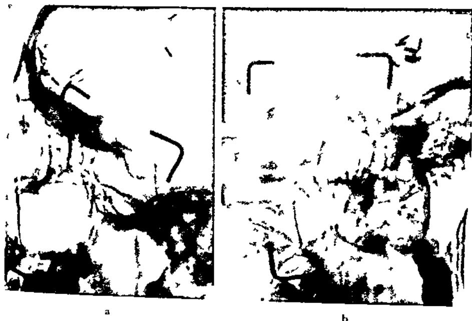


Fig 2 a) Simulator roentgenogram One of the lateral fields (markers) with the pituitary fossa b) The right anterior field (markers) in the same patient

et coll 1971) and consisted of a four field technique with two lateral and two anterior fields, the latter deflected about 15° inwards, the radiation energy was 190 kVp with a HVL of 1 mm Cu (Fig 1) The eye was shielded with lead during the treatment to the anterior portal The target area always included the base of the skull around the sella turcica The estimated tumour dose to the nasopharynx ranged roughly from 5 000 to 6 000 rad If the neoplasm was still present 6 to 8 weeks after completion of the external radiation course, a 50 mg radium source was applied locally on one or two occasions for 3 to 8 hours Irradiation with from 1 200 to 3 300 R was applied to the neck whenever the nod-
tr 16 of the 29 patients received

Theoretic reconstruction of dose distribution An attempt was made to reconstruct the dose distribution for 20 patients individually Simulator roentgenograms were obtained of all fields demonstrating the presence of the pituitary fossa within the boundaries (Fig 2) As regards the anterior portal, the sella turcica was recognized in most simulator films near the inner margin of the field and close to the lower border of the eye shield (Fig 3) Contours were

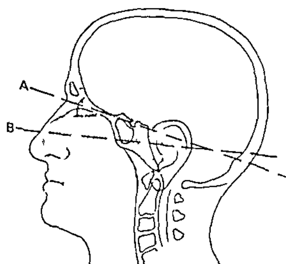


Fig 1 Boundaries of the anterior and lateral fields A The plane of the isodose plotting B The approximate direction of the a p beams

1957) or by ^{60}Co gamma beams (BACKLUND et coll 1972) with the specific intention of destroying the pituitary gland partly or completely

The intention of the present work was to investigate the possibility of any late effects on the pituitary gland of external radiation therapy procedures for non pituitary, extracranial disorders A group of patients treated for nasopharyngeal tumours was selected The centre of the middle cranial fossa, including the sella turcica, is usually part of the target area because of the tendency of the tumour to invade the base of the skull Patients surviving for some years after treatment almost never have involvement of the base of the skull with the risk of an intracranial spread of the tumour They therefore constitute suitable subjects for the examination of possible late radiation induced pituitary changes

Material A group of 58 patients, alive at least 10 years after treatment for nasopharyngeal malignancy, was selected Twenty-nine of these were eliminated for the following reasons four patients were over 80 years of age, 3 patients had mental disorders of non endocrine origin and one patient was under cortisone treatment for severe rheumatoid arthritis (No other hormone was given) The remaining 21 patients refused to submit to the time-consuming examination because they were doing well and had full-time occupations The total number of patients investigated was 29 (9 women and 20 men), the case records of the remaining, approximately 620 patients, treated for nasopharyngeal tumours (observation time 5 to 30 years) were reviewed for any observations on possible pituitary insufficiency or on hormone therapy

Method This was described in detail in a previous paper (DE SCHRYVER

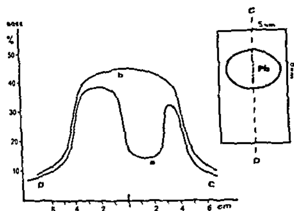


Fig 5 Variation of radiation intensity (percentage of surface value) at 7 cm depth as a function of chamber position a) With and b) without lead shield

fore considered that two estimates should be calculated a minimal, based on the assumption that the pituitary gland had been wholly shielded during the entire treatment course, and a maximal where the reverse was presumed The dose values thus obtained were then added to the lateral field contribution computed diagrammatically to yield the range of the total pituitary tissue dose

Phantom measurements As a check on the diagrammatic dose reconstructions of the lateral field contributions as well as a means of obtaining data on the dose contribution from the anterior fields, phantom measurements were made both in a water and an anatomic skull phantom, previously described in detail (DE SCHRYVER et coll 1971) For the lateral field contributions, these measurements presented good agreement with the values obtained diagrammatically The anterior field contribution varied between 12 per cent (behind lead protection) and 38 per cent (without lead protection) of the nominal surface dose (Fig 5) These values were then added to the lateral field contributions, as determined from the isodose reconstructions, to obtain the range of the actual total dose in the pituitary region As regards the dose contributions to the sella turcica from the radium applications, it should be borne in mind that it was impossible to know in retrospect exactly where in the nasopharyngeal cavity the applicator was located Yet, as the flat part of the absorption curve is being dealt with it appeared that the dose at the sella turcica could be estimated at approximately 30 + 5 rad h Among the 20 patients for whom dose calculations were available 15 had radium applications with doses to the sella turcica ranging from 120 to 480 rad Dose calculations were not available for 9 of the patients, however as these failed to constitute a selected group, either clinically or with regard to the treatment technique, their data were included in the investigation



Fig 3 Simulator roentgenogram of the right anterior field in a skull phantom with the position of the pituitary gland (marker) in its fossa

obtained of the skull approximately at the level of the sella turcica, the position of the treatment fields reproduced, and the dose contributions from the lateral portals plotted with standard isodose diagrams (Fig 4)

The presence of eye-shields suggested that the dose contribution from the anterior portals would be assessed more properly by phantom measurements (see below) Furthermore, irradiation was being given, at that time, without help of any head-immobilizing device and slight movement during treatment or any variation in positioning from session to session could have resulted in the pituitary gland being partly or wholly shielded during part of the treatment It was there-

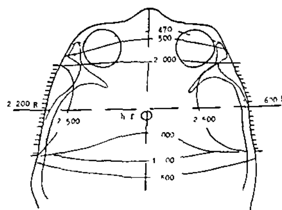


Fig 4 Example of computed dose distribution in plane A (lateral field contributions only) h f = hypophyseal fossa

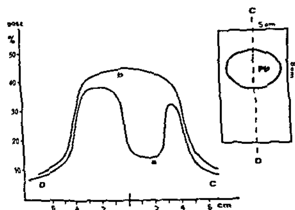


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Table 1

A Cases without clinical or laboratory signs of disturbed pituitary function B Cases in which the laboratory examination failed to rule out disturbed pituitary function

	Case	Sex	Pres Age	Years after treat	Age at treat	Estim dose (rad) to gland (incl Ra contr)	Ext treat time in weeks
A	50	M	43	20	22	2 400 – 3 700	5
	51	M	67	15	51	1 800 – 3 800	4
	57	M	50	13	36	–	–
	59	M	63	27	35	3 600 – 5 200	5 5+2 5
	60	M	43	27	16	2 300 – 3 100	3
	62	M	63	25	38	4 100 – 5 400	6+1
	134	F	64	18	45	3 500 – 5 000	4
	135	F	60	19	41	3 100 – 4 500	5
	137	F	77	22	54	2 700 – 3 800	3
	142	M	68	27	41	3 000 – 4 300	6
	146	M	69	21	47	1 700 1 700	3
	147	M	74	22	52	2 800 – 4 100	4 5
	148	M	63	26	36	3 300 4 200	5
	151	M	77	30	47	1 700 2 400	5
	152	M	57	28	28		–
	153	F	61	12	48	3 300 4 700	4
	160	M	63	18	46		–
	161	F	62	17	44		
	163	F	65	17	48	3 200 4 700	4 5
	166	M	52	15	37	3 000 4 400	4
	168	F	56	10	45	–	–
	171	M	48	12	36	–	
	172	F	48	11	37	–	
B	48	M	55	21	34	5 100 6 800	5 5+3
	136	M	62	19	43	4 000 5 400	5 5
	164	M	67	15	52	–	
	165	M	75	15	59	3 100 – 4 500	4
	170	M	56	11	45		–
	173	F	61	11	49	2 900 4 300	4

Assessment of pituitary function The patients were examined by a careful evaluation of the history, clinical features and laboratory investigations The latter included the protein bound iodine (PBI, normal range 4 0 to 8 0 μg/100 ml), T_3-resin uptake test (normal values 25 to 35 per cent), serum cholesterol (normal values 150 to 300 mg per cent), thyroid uptake of radioiodine (normal

values 20 to 50 per cent uptake after 24 h) and the metyrapone test. The metyrapone test was performed when the patients were in hospital by the collection of 24-h urine samples for five consecutive days. The 17-ketogenic steroids (17 KGS) of the urines were assayed by the method of APPLEBY et coll. (1955). On the third day 0.75 g metyrapone was given orally every fourth hour. The response of the urinary 17-ketogenic steroids was expressed as the maximal increase as a percentage of the mean basal value, an increase of 100 per cent or more was regarded as a normal response, provided the basal level was also within normal limits. Unfortunately, no radioimmunoassay method for the determination of the gonadotrophins was available at the time of the investigation. Two patients were, moreover, evaluated by the TRH-stimulation test, this test, based upon the intravenous administration of 50 μg TRH (= thyrotrophin releasing hormone, synthetic peptide, F. Hoffmann-La Roche & Co., A.G.) and radioimmunoassay for human thyrotrophin was performed as described by KARLBERG et coll. (1971).

Results

Estimated absorbed radiation doses. The tissue doses in the sella turcica, as computed and checked by the techniques described, ranged from 1 700 to 5 100 rad (mean 3 080 ± 176) for the lowest and from 1 700 to 6 800 rad (mean 4 300 ± 247) for the highest estimates (all doses rounded off to the nearest hundred).

Endocrinologic data. Twenty-three of the 29 patients had no clinical or laboratory signs of any disturbance in the hypothalamic-pituitary system as judged by the parameters tested. Sex, age, years after treatment, estimated dose at the pituitary gland and duration of treatment for these patients are presented in Table 1. None of the remaining 6 patients had any history or clinical signs of endocrine insufficiency but the laboratory data were insufficient for technical reasons in 3 and could indicate the presence of a disturbed hypothalamic pituitary function in the 3 remaining patients. The results from the laboratory investigations are presented in Table 2.

Case 48 had an uptake of radioiodine below the normal limit. The results from the metyrapone test revealed low basal values for the excretion of the 17-KGS but an increase after the administration of metyrapone. The patient was recalled and the tests repeated with the addition of a TRH stimulation test, this time all tests but the 24-h uptake test were normal. No explanation for the low radioiodine uptake was possible. A pituitary lesion was improbable in view of the normal response to metyrapone and TRH, nor was a lesion in the hypothalamic region compatible with the normal metyrapone test. Primary hypothyroidism

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	142	M	68	27	41	3 000 4 300	6
	146	M	69	21	47	1 700 1 700	3
	147	M	74	22	52	2 800 4 100	4 5
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	161	F	62	17	44		–
	163	F	65	17	48	3 200 4 700	4 5
	166	M	52	15	37	3 000 4 400	4
	168	F	56	10	45		–
	171	M	48	12	36		
	172	F	48	11	37		
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	51	M	67	15	51	1 800–3 800	4
	57	M	50	13	36	–	–
	59	M	63	27	35	3 600–5 200	5 5+2 5
	60	M	43	27	16	2 300–3 100	3
	62	M	63	25	38	4 100–5 400	6+1
	134	F	64	18	45	3 500–5 000	4
	135	F	60	19	41	3 100–4 500	5
	137	F	77	22	54	2 700–3 800	3
	142	M	68	27	41	3 000–4 300	6
	146	M	69	21	47	1 700–1 700	3
	147	M	74	22	52	2 800–4 100	4 5
	148	M	63	26	36	3 300–4 200	5
	151	M	77	30	47	1 700–2 400	5
	152	M	57	28	28	–	–
	153	F	61	12	48	3 300–4 700	4
	160	M	63	18	46	–	
	161	F	62	17	44	–	
	163	F	65	17	48	3 200–4 700	4 5
	166	M	52	15	37	3 000 4 400	4
	168	F	56	10	45	–	
	171	M	48	12	36	–	–
	172	F	48	11	37		–
B	48	M	55	21	34	5 100 6 800	5 5+3
	136	M	62	19	43	4 000–5 400	5 5
	164	M	67	15	52		–
	165	M	75	15	59	3 100–4 500	4
	170	M	56	11	45		–
	173	F	61	11	49	2 900–4 300	4

Assessment of pituitary function The patients were examined by a careful evaluation of the history, clinical features and laboratory investigations The latter included the protein bound iodine (PBI, normal range 4 0 to 8 0 μg/100 ml), T_3 resin uptake test (normal values 25 to 35 per cent), serum cholesterol (normal values 150 to 300 mg per cent), thyroid uptake of radioiodine (normal

ment in the hypothalamo-pituitary adrenal system Whether this was due to the patients age (75 years) or to irradiation could not be determined The first alternative seemed more probable as the thyroid function tests were normal as was the clinical examination The response to an oral dose of metyrapone in old age can be decreased without any indication of disease (KAALUND-JENSEN & BLICKERT TOFT 1970) The patient was marked for reexamination but had meanwhile died from a gastrointestinal haemorrhage

Case 170 had a 24 h uptake of radioiodine which was on the border line (20 per cent) to a low value The other laboratory parameters were normal and the patient was regarded as having no endocrine insufficiency

Case 173 had a low 24 h uptake of radioiodine The basal level for the excretion of the 17 KGS was in the normal range but the effect of metyrapone could not be fully evaluated due to incomplete results The patient was re-investigated with the addition of the TRH stimulation test This time the uptake of radioiodine was on the borderline to low values A subnormal increase of 17 KGS excretion after metyrapone administration was obtained TSH increased after TRH administration but was also close to the lower borderline Thus, a slight impairment of the hypothalamo-pituitary system could not be excluded The patient refused further examination as she felt perfectly well

The present investigation did not afford any evidence for pituitary insufficiency after external radiation therapy with a pituitary dose of up to 6 800 rad This was judged by the screening of 620 other case records for patients treated for nasopharyngeal tumours (The exception was a patient who had right sixth cranial nerve palsy without radiologic signs of involvement of the base of the skull on admission but who developed diabetes insipidus 1 year after treatment)

Discussion

Hypopituitarism as a result of external irradiation procedures seems to be rare and only a few cases have been reported TAN & KUNARATNAM (1966) described a case of pituitary dwarfism developing in a girl treated for nasopharyngeal carcinoma at the age of 12 years The total tumour dose amounted to 9 300 rad divided into two courses Radiation therapy caused amenorrhea and an arrest in growth At the age of 23 she was reexamined and hypogonadism as well as low excretion of 17 keto-and 17 hydroxycorticoids were evident Replacement therapy was initiated with marked improvement, no evidence of malignancy

BRADLEY (1965) described a 50 year old patient, given approximately 6 000 rad tumour dose in 2 courses for anaplastic carcinoma of the nasopharynx The patient eventually developed signs of hypopituitarism clinically manifest 7 years after the treatment This case is somewhat more dubious as there was already

Table 2

Laboratory data from the six cases with pituitary function possibly disturbed

Case	18		136	164	165	170	173	
	1st	2nd					1st	2nd
PBI, μg/100 ml	5 0	5 6	8 6	7 0	6 8	4 8	7 9	6 6
T_3 resin, %	29	25	32	30	29	34	31	28
Cholesterol, mg %	315	290	169	199	381	209	237	210
24 h ^{131}I uptake, %	16	10	0 8	25	28	20	3	23
PB ^{131}I exer., %	50	63	61	33	31	46	69	66
17 KGS mg/24 h, Day 1	2 9	11 6	13 1	—	16 3	—	8 2	7 6
(g creatinine/24 h)	(0 8)	(—)	(1 5)		(—)		(0 6)	(—)
Day 2	*1 8*	*8 0*	*5 2*	—	*4 6*	*13 0*	*11 1*	*6 2*
	(0 9)	(1 3)	(1 1)		(—)	(1 3)	(0 8)	(0 7)
Day 3	—	11 8	11 2	8 0	7 8	11 0	14 6	9 6
	(1 2)	(1 1)	(1 0)	(1 0)	(1 1)	(1 2)	(0 9)	(1 0)
Day 4	16 4	29 6	16 1		7 5	19 4	—	11 4
	(1 7)	(—)	(—)		(0 9)	(1 1)	(0 4)	(0 8)
Day 5	—	19 3	11 5	—	7 1	26 2	—	11 3
	(1 6)	(1 6)	(0 9)		(1 0)	(1 2)	(0 8)	(0 9)
TSH, μU/ml	—	9		—	—	—	—	12 5
TSH after TRH stim., μU/ml	—	*20 5*	—	—		—	—	*23*

was also unlikely because of the normal basal values for the TSH, PBI and T3-resin test An iodine block was possible, although, because of the normal PBI, doubtful *In summary, decreased function in the hypothalamo-pituitary system* could not be excluded but was unlikely

Case 136 had been on thyroid medication (*0 3 mg l-thyroxine daily*) for six years No pre-treatment data on thyroid function were available The treatment was initiated because of fatigue which eventually disappeared during medication The excretion of 17-KGS after metyrapone administration increased although this was somewhat difficult to evaluate because of a missing creatinine value The patient was recalled for further examination, this was refused Impaired function of the hypothalamo-pituitary system was unlikely

Case 164 had thyroid function tests within normal limits *The metyrapone* test was incomplete and could not be evaluated The patient refused reexamination because he felt well In summary, *impaired function in the hypothalamo-pituitary* system was unlikely

Case 165 had thyroid function tests within normal limits *However, no in*crease in the excretion of the 17-KGS steroids was obtained, indicating impair-

ment in the hypothalamo-pituitary adrenal system Whether this was due to the patients age (75 years) or to irradiation could not be determined The first alternative seemed more probable as the thyroid function tests were normal as was the clinical examination The response to an oral dose of metyrapone in old age can be decreased without any indication of disease (KAALUND-JENSEN & BLICKERT TOFT 1970) The patient was marked for reexamination but had meanwhile died from a gastrointestinal haemorrhage

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diplopia on admission with the possibility of involvement of the base of the skull. No radiologic examinations were reported.

LEDERMAN (1961) failed to observe a single instance of pituitary insufficiency after treatment in a series of 241 patients with nasopharyngeal tumours treated with irradiation that included the pituitary. The observation time was from 2 to 27 years.

The target dose given under these circumstances by external radiation is almost always well under 10 000 rad. With higher doses the hazards of serious damage to the surrounding tissues increase. The doses needed to abolish the function of the normal human pituitary gland may be evaluated by the special techniques with heavy particle or Cobalt 60 beams or radioactive implants with the specific aim of completely or partly destroying the pituitary gland.

McCOMBS (1957) stated that with doses under 20 000 rad there was only infrequent histologic evidence of necrosis, with doses of more than 20 000 rad, damage occurred in all patients, a dose of 30 000 rad caused almost complete necrosis. Recent observations by BACKLUND et coll. (1972) indicated that the zone of necrosis in a pituitary gland treated by external ^{60}Co gamma radiation was confined within an area that received 18.5 ± 1.5 krad. Estimates based on an examination of implanted glands are usually higher. RASMUSSEN (1953) reported in patients treated with ^{90}Y implants that doses of about 110 000 to 190 000 rep were required to cause destruction of the pituitary gland. NOTTER (1959) gave the threshold dose necessary to produce necrosis as in the range of 55 000 to 120 000 (mean 90 000) rad. YOUNG (1957) reported the threshold dose under similar conditions to be around 70 000 rad. It should, moreover, be remembered that in all instances quoted, the dose was given in a non-fractionated way and that full destruction was the aim. This would not necessarily preclude the possibility that lower doses might still injure the gland. However, KELLY et coll. (1951) discovered no indication of pituitary injury in 3 patients given 8 100 to 10 000 R to the pituitary gland by means of a multiple-port coronal technique, the observation time for these patients was only 3, 4.5 and 12 months respectively. PLUNKETT (1957) noticed a transitory decrease in the 24 h ^{131}I-uptake in the thyroid in 8 out of 12 patients treated with 4 000 a 6 500 R to the pituitary gland over 3 weeks (^{60}Co gamma radiation) for disseminated breast or prostatic carcinoma. Four out of 10 patients had some decrease in the urinary gonadotrophin excretion, the observation time was 1 to 13 months.

Numerous animal experiments have been performed, thorough discussion of the results appearing in the review by RUBIN & CASARETT (1968). As much of this work refers to single dose schedules and as the sensitivity to irradiation may be expected to vary between different species, any conclusion with regard to the human pituitary would however seem difficult.

No investigations in which pituitary function was tested by methods to evaluate the reserve capacity of the normal human gland a long time after radiation therapy, appear to have been published. This may be relevant, as pointed out by LOWRY (1970) who recently discussed a possible late effect of irradiation,

primary hypothyroidism, none needed replacement therapy, nor had any patient clinical signs of pituitary insufficiency. The laboratory tests could for practical reasons not be extended in a few patients in whom the reserve capacity of the pituitary gland was not fully evaluated and in whom a small decrease could not have been ruled out with certainty.

The conclusion must thus be reached that the normal human pituitary gland may be irradiated with a dose up to at least 5 000 rad over 6 weeks without risk of ensuing late endocrine insufficiency of any clinical importance.

SUMMARY

Twenty nine patients who had received radiation therapy 10 to 30 years previously for nasopharyngeal tumours were assessed both clinically and by laboratory examinations for a possible late radiation induced impairment of pituitary function. The radiation doses administered (1 700 to 6 800 rad) produced no evidence of pituitary insufficiency nor did any patient need replacement therapy.

ZUSAMMENFASSUNG

Neunundzwanzig Patienten, die 10 bis 30 Jahre zuvor wegen eines Nasopharyngeal Tumors eine Strahlentherapie erhalten hatten, wurden hinsichtlich einer möglichen späten, durch Strahlung hervorgerufenen Beeinträchtigung der Hypophysen Funktion sowohl klinisch als auch mit Laboratoriumsmethoden untersucht. Die verabfolgte Strahlendosis (1 700 bis 6 800 rad) hatte keine Zeichen einer Hypophysen Insuffizienz zur Folge und keiner der Patienten benötigte eine Substitutionstherapie.

RÉSUMÉ

Vingt neuf malades qui avaient été traités par les radiations il y a 10 à 30 ans pour des tumeurs nasopharyngiennes ont subi des examens cliniques et de laboratoire pour rechercher l'éventualité d'un trouble tardif de la fonction hypophysaire dû aux radiations. Les doses de radiation administrées (1 700 à 6 800 rad) n'ont pas causé de signe d'insuffisance hypophysaire et aucun malade n'avait besoin d'un traitement substitutif.

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THE DISTRIBUTION OF ^{198}Au INJECTED INTRAVENOUSLY AS A COLLOID AND IN SOLUTION

N-E SATERBORG

Colloidal radiogold (^{198}Au) has been used extensively in clinical examinations of the reticulo-endothelial system It is, like other colloidal materials after intravenous injection, mainly taken up by the liver, spleen and bone marrow The colloid has been most widely used for liver scanning (FRIEDELL et coll 1957, and others) but the relatively small part taken up by the bone marrow may also be utilized for examinations in vivo Bone marrow scintigraphy was described by LARSSON & JONSSON (1957) and has become useful in the examination of active bone marrow (ENGSTEDT et coll 1958, LARSSON et coll 1960, HOFER & EGERT 1963, EDWARDS et coll 1964, KNISELEY et coll 1964, KNISELEY 1972)

This and a number of ensuing articles will present certain experimental findings with a view further to elucidate the distribution of colloidal ^{198}Au in the bone marrow after its intravenous injection Several radioactive elements in colloidal form appear to become soluble and so liberate part of their activity, this of course influences the distribution of the injected radionuclide, as for instance with radiation active colloidal manganese (SHEPPARD et coll 1947, HAHN & CAROTHERS 1950) and colloidal ^{90}Y-fluorid (LAHR et coll 1955) The possibility of whether some part of radiogold, injected as colloid, behaves as soluble radiogold has now been investigated The behaviour of radiogold in this respect is of importance for the interpretation of clinical bone marrow scintigraphy

Submitted for publication 24 April 1973

Gold salts, such as gold chloride, are rather unstable in solution and have a tendency to be hydrolyzed to particulate $Au(OH)_3$ (Svedberg 1925). Injected into the blood soluble gold reacts chemically with different constituents of the plasma (Issekutz & Dirner 1930, Simon 1954, Murray 1956, Coke 1963, Cohen & Rousselet 1969 and Eberl & Altmann 1970). A survey of the chemistry, pharmacology and pharmacy of gold compounds was presented by Nineham (1963).

Investigations of the distribution of soluble gold injected into the organism were earlier mainly motivated by gold therapy in rheumatoid arthritis and tuberculosis. Experimental observations were obtained by histochemical methods (Christeller 1927, Leitman et coll. 1946, Block et coll. 1942, Okkels 1930, Harding 1953), by chemical analyses of the gold content of different organs or excretions (Freyberg et coll. 1942, Block et coll. 1942, 1944) or by the estimation of the radiation activity in different organs after the intramuscular, intravenous or oral administration of radioactive gold compounds (Jeffrey et coll. 1958, Bertrand et coll. 1948, Kleinsorge et coll. 1959, Lawrence 1961).

Most earlier investigations of the distribution and excretion of gold compound were performed after their oral or intramuscular administration, and little work on their intravenous injection has been reported. Bertrand et coll. (1948) examined the organ distribution of ^{198}Au in rats and rabbits two to eight days after the intravenous injection of gold sodium thiosulfate. Irrespective of the route of administration, soluble gold usually accumulated mostly in the kidneys but also in the spleen and liver (Block et coll. 1941, 1942, 1944, Bertrand et coll. 1948). Histochemically it was found that the accumulation of soluble gold after its injection took place in the proximal convoluted tubules of the kidneys, in the cells of the red pulp of the spleen and in the Kupffer cells of the liver. The distribution was greatly influenced quantitatively by the gold compound used (Block et coll. 1944). The gold was mainly excreted in the urine but the excretion was slow, ranging for example between 15 and 25 per cent during the first two weeks after an intramuscular or intravenous injection (Bertrand et coll. 1948, Jeffrey et coll. 1958, Lawrence 1961).

Soluble gold may easily be converted to colloid by means of reducing processes. "Aurum potabile" of the alchemists was a colloidal gold sol processed by reducing a gold solution with etheral oils. Glucose was used as a reducing substance in the method described by Del Turco & Pietra (1960) and particles processed by neutralizing gold chloride with NaOH served as nuclei for the formation of colloidal gold particles.

The distribution in the body of all colloidal material after its intravenous injection is almost always the same with the largest accumulation in the liver, spleen and bone marrow. The particle size of the colloid influences, however

the relative distribution between different reticulo-endothelial organs (DOBSON et coll 1949, 1952, ZILVERSMIT et coll 1952) The distribution of colloidal radiogold has been extensively investigated both in animals (SHEPPARD et coll 1950, WILLIAMS et coll 1950, BERG 1951, ZILVERSMIT et coll 1952, GANZ & BRUCER 1958) and in man (SHEPPARD et coll 1947, ROOT et coll 1954) The changing distribution of colloidal gold at different times after its intravenous injection has also been demonstrated by autoradiography of single organs (STEMBRIDGE et coll 1953)

The distribution of radiation activity after intravenous injections of colloidal and soluble ^{198}Au in mice has now been investigated by means of whole body autoradiography and micro-autoradiography of excised organs Measurements of activity in the blood urine, faeces and the excised organs were also performed No comparison of the distribution at varying times after the intravenous injection of colloidal and soluble gold nor the changing distribution during the first 24 hours after the injection of soluble gold appears previously to have been reported

Material and Methods

Adult Swiss albino mice were used, whether the animals were pregnant or not was of no real significance, and no distinction was therefore made on that account The ^{198}Au was used as a colloid or in solution for the injections and some plasma samples were examined by means of ascending chromatography in 95 per cent methanol on thin layer plates (TCL plates, Silicagel, Merck) The activity was measured by an automatic radiation chromatogram scanner (beta method)

^{198}Au colloid The colloid (AB Atomenergi, Studsvik) was produced by the method of DEL TURCO & PIETRA (1960) This colloid has a fairly uniform particle size with a diameter of 250 ± 50 Å In chromatograms of most newly processed ^{198}Au sols the activity remained at the site of application The chromatographic result was unchanged in repeated examinations of such sols up to 30 days after processing The activity in the chromatograms in some newly-processed sols was however divided and located partly at the site of application and partly at the solvent front migrating as soluble gold These sols were excluded from the investigation

^{198}Au solution Five different radioactive gold chloride solutions, commercially available (AB Atomenergi, Studsvik), were examined by chromatography from five to fifteen days after processing The relative amount of activity remaining at origin (probably colloid) varied from one to 50 per cent Only solutions from

which less than four per cent of the activity remained at origin were accepted for use. By adding veronal buffer solution pH 8.6 to the acid gold chloride solution (pH less than 0.5) a final solution of unknown chemical composition of pH 4.0 was produced. Less than four per cent of the activity remained at origin in the ascending chromatograms of this solution; the solution was always used immediately after processing.

Autoradiography was carried out in 27 adult mice. Fifteen mice were injected with colloidal ^{198}Au and 9 with ^{198}Au solution; the animals were killed for serial sectioning at 10, 30 and 60 minutes and 3, 6 and 24 hours. One additional animal was killed at 72 hours in the series injected with ^{198}Au solution and in the colloid series two additional animals were killed at 2 and 48 hours. The activity injected was about 1 mCi. The volume of colloid varied between 0.1 to 0.5 ml and that of the ^{198}Au solution between 0.1 to 0.3 ml. The administration of greater volumes of the ^{198}Au solution caused rapid death of the animals from toxic effects. The animals were killed by immersion in a solution of solid carbon dioxide in n-hexane. An autographic technique described by ULLBERG et coll. (1954, 1969) was used. Sagittal sections 20 μ thick were prepared from the animals at various levels, the sectioning, drying and autoradiographic exposures being carried out in a freezing box at —20° C on Structurix film (Agfa-Gevaert). Each section was placed between two films, one of which was used as a control for arriving at an appropriate exposure — usually 3 to 15 days. Three animals were used as controls; these were injected with saline in place of ^{198}Au but were then treated like the other animals. Two of the control animals were killed at 2 hours and one at 24 hours. The intention was to compare the uptake of ^{198}Au in organs with a low uptake and those with a high uptake. In some autoradiograms therefore heavy overexposure had to be accepted for organs with a high uptake in order to define the uptake in organs with low uptake. This of course reduced the possibility of using the films for semiquantitative interpretation. Quantitative data were, however, obtained from direct measurements of the activity in excised organs in some animals. Two hours after the injection of the colloid or the solution the distribution of the ^{198}Au in the liver, spleen, bone marrow and kidneys was examined by micro-autoradiography; this was performed with the stripping film method on paraffin-embedded tissue specimens. A brief description of methods available for autoradiography was made by FITZGERALD et coll. (1953); more detailed descriptions of different autoradiographic techniques were published by BOYD (1955) and ROGERS (1967).

Measurements in a well-scintillation counter. Repeated blood samples were taken in some animals at different times after the injection and measured in a well scintillation counter. These samples obtained three minutes after the injection

of the colloid or the solution were centrifugated at 2 000 rpm for 4 minutes. The radiation active material in the plasma from some of these samples was examined by radiochromatography. Some other centrifugated blood samples were frozen in the capillary tubes and placed on an ordinary film for exposure over a period of four days.

Four animals were injected with ^{198}Au solution and three with ^{198}Au colloid, each injection being of 0.15 mCi. The animals were placed in metabolic cages and the excretion collected on filter paper; the faecal excretion was mechanically removed from the paper at 24 and 48 hours. The activity in the faecal excretion and in the filter paper, the latter mainly containing activity from the urine, was measured separately in a well scintillation counter. Some urine samples were examined by radiochromatography.

Two series of animals were injected with 0.15 mCi ^{198}Au colloid and solution respectively. Two animals from each series were killed at 2 hours, 24 hours and 7 days. The heart was opened and a syringe placed into the ascending aorta and the inferior vena cava cut. The arterial and venous systems were perfused with saline, the perfusion continuing until pure saline seemed to come from the vein. The liver, kidneys, spleen and femur were removed and liquified in concentrated hydrochloric acid; the activity was measured in a well scintillation counter and compared with a standard activity of ^{198}Au.

Comparisons of mean values were made by using Wilcoxon's test for unpaired observations.

Results

The activity diminished rapidly in the blood after the injection of the colloid and most had disappeared after only 10 minutes. The activity diminished more slowly after the injection of the ^{198}Au solution except for the first minutes of distribution, and remained after about 10 minutes at an almost constant level that was still relatively high compared with that during the first minutes. The blood clearance curves appear in Fig. 1 and are compared with a curve obtained after the injection of ^{131}I labelled serum albumin (RISA). All activity remained at origin in chromatograms of plasma at three minutes after injection of ^{198}Au colloid and thus probably represented colloid (Fig. 2), while after injection of ^{198}Au solution the result was more variable with some activity remaining at origin and some migrating as soluble gold. In the capillary tubes with centrifuged blood the radiation activity after injection of ^{198}Au colloid was mainly in the layer of packed white blood cells and thrombocytes, while some also lay in the serum layer. Only a minute amount of activity was present in the layer of packed red

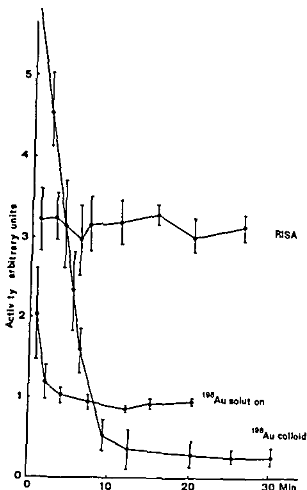


Fig. 1. Concentration of activity in the blood at varying times after the intravenous injection of ^{198}Au solution, ^{198}Au colloid and ^{131}I labelled serum albumin.

blood cells. After the injection of ^{198}Au solution most of the activity was in the serum layer.

After the colloid injection both the urinary and faecal excretion of ^{198}Au was small, while during the first two days after the solution the excretion was considerably larger (Table 1). Chromatography revealed that ^{198}Au in the urine after the injection of both colloid and solution behaved as the former (Fig. 3).

The concentrations of ^{198}Au in the excised liver, spleen, femur and kidneys were very different after the injection of the colloid and the solution. The concentrations in the liver and spleen were much lower after the injection of the latter than after the injection of the colloid ($p < 0.01$). The injection of the solution produced after 2 hours up to fifty times larger concentrations of ^{198}Au in the kidneys than the injection of the colloid, the concentrations of ^{198}Au in the femur were after the same time two to three times larger after the injection of colloid than after the solution. The concentrations of ^{198}Au in the liver after

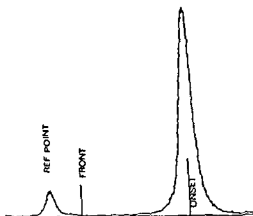


Fig 2 Thin layer chromatography of plasma collected 3 min after intravenous injection of colloidal ^{198}Au in a mouse

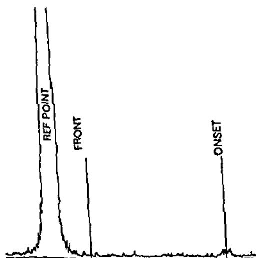


Fig 3 Thin layer chromatography of urine collected from a mouse during 24 hours after intravenous injection of colloidal ^{198}Au

the injection of colloid decreased with time ($p < 0.05$) The kidney concentrations of ^{198}Au after the injection of solution also decreased with time ($p < 0.01$). The results are summarised in Table 2 and graphically registered in F ~ 4

Th

Th more slowly in the spleen and bone marrow although after about 30 minutes had reached the same order of magnitude as in the liver The high concentrations in the liver, spleen and bone marrow then persisted during the whole examination (up to 48 hours). The distribution after ten minutes, one hour, and twenty-four hours appear in Figs 5 and 6 A slight

Table 1

Excretion of ¹⁹⁸Au after intravenous injection of ¹⁹⁸Au colloid and ¹⁹⁸Au solution expressed as percentages of the activity injected

	^{198}Au solution		^{198}Au colloid	
	24 hour excretion	48 hour excretion	24 hour excretion	48 hour excretion
Urine	13 1	16 8	0 05	0 08
	13 5	18 2	0 04	0 07
	15 0	23 3	0 05	0 08
	26 5	33 8	0 05	0 08
Faeces	1 3	2 9	0 02	0 06
	1 2	3 2	0 02	0 07
	2 3	4 0	0 03	0 07
	2 9	5 7		

accumulation also appeared during the first few hours in the kidneys and continued up to 48 hours An uptake of ^{198}Au was observed in the adrenals localised to the deep cortex zone, it was most marked after 10 minutes but could still be observed at 24 hours The concentration of ^{198}Au in the lungs was low, except at the first observation at 10 minutes, it was, however, at all times low compared with the concentration in the other regions The intestinal wall was at 24 hours outlined with activity in the visceral peritoneum, none was evident in the gastric or intestinal mucosa or in the pancreas Some concentration was evident in the amnion-epithelium of the pregnant mice

The distribution of activity after injection of soluble ^{198}Au followed a markedly different pattern A relatively high concentration could still be registered in the blood at 10 minutes, and in the autoradiograms, for instance, was visible in the blood pool of the heart A high concentration was also observed in the kidneys and lungs The concentration in the latter then slowly decreased, was low at 24 hours and was not visible at 72 hours The concentration in the kidneys increased, however, continuously with time after the injection in relation to other organs At 72 hours the highest concentrations of ^{198}Au were in the kidneys, spleen and liver The concentration in the bone marrow was low A definite concentration was evident in the amnion epithelium in the pregnant animals, a concentration was also observed in the placenta and at 72 hours had reached the same order of magnitude as in the liver

A section of the femur was examined with the same technique as used for whole body autoradiography, this demonstrated that the low activity observed in the skeleton after injection of the solution emanated from the bone marrow

Table 2

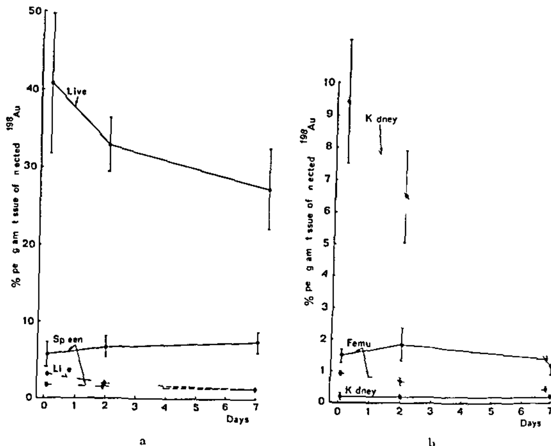
The quantitative distribution of ^{199}Au in liver, spleen, femur and kidney 1 and 24 hours and 7 days after injection of ^{199}Au colloid or ^{199}Au solution (neutralised gold chloride) in mice. The values are expressed in means as percentages per gram tissue of the activity injected

	2 hours		24 hours		7 days	
	Colloid	Solution	Colloid	Solution	Colloid	Solution
Liver	40 8±9 0 n = 5	3 0±0 3 n = 10	32 9±3 3 n = 8	1 9±0 3 n = 5	27 2±5 2 n = 5	1 3±0 2 n = 4
Spleen	5 8±1 6 n = 5	1 6±0 1 n = 10	6 7±1 3 n = 8	1 5±0 3 n = 5	7 4±1 3 n = 5	1 2±0 3 n = 4
Kidney	0 2±0 1 n = 5	9 4±1 9 n = 10	0 14±0 03 n = 8	6 5±1 4 n = 5	0 2±0 1 n = 5	1 2±0 3 n = 4
Femur	1 5±0 2 n = 5	0 9±0 1 n = 10	1 8±0 5 n = 8	0 7±0 1 n = 5	1 4±0 2 n = 5	0 4±0 1 n = 4

The distribution of activity in the liver, spleen and bone marrow in micro-autoradiograms from organs excised after three hours revealed in the main the same pattern after the injection of ^{199}Au colloid and solution, despite the large quantitative differences (Figs 7, 8) In the liver the ^{199}Au was located to the Kupffer cells in the periportal regions and in the spleen it lay in the red pulp, it presented a spotty distribution in the bone marrow, corresponding well to an uptake in the endothelium of the sinusoids A marked difference was evident in the kidneys in the distribution after the injection of colloid and solution After the former the activity was entirely concentrated to the glomeruli with no detectable amount in the tubuli After the injection of the solution the activity lay in the proximal parts of the tubuli with none in the glomeruli

Discussion

The present investigation confirms in the main earlier experiences in the behaviour of [illegible] ary in order to a [illegible] ^{199}Au sols before [illegible] materials delivered a considerable amount of ^{199}Au proved to be in soluble form If this were rectified, however, the colloid seemed to be stable both in vitro and in vivo No indications were apparent that any significant amount of the ^{199}Au colloid injected was transformed in vivo into soluble ^{199}Au The blood after the injection was rapidly cleared from ^{199}Au through reticulo-endothelial activity and only small amounts were excreted in the



Γ g 1 Tl e relat e a ou t of ^{198}A per gram t ss e a) l er and splee an l b) k dney a d fem r 2 l ours 24 l o rs a d 7 lays after ntra e o s nject ons of ^{198}A sol t o (ne tral ed gold cl lor de) ( ) and ^{198}A collo l (——)

Γ g 3 A tora l ogr ms of tl ree ce k lled at a o s t es after tl e intra e o s njec o of col o dal ^{198}A a) At ten n tes Cons derable act ty pers sed n tl e re la ng blood as e de ced by tl e blood pool i tl e l ea t 5) an l l ger blood e sels 12) He up ke n tl e l er (3) an l l gs 4 b t o ly sl gl t uptake n tl e spleen (1) a d e el ae 8 Tl e suprarenal glands co entrated act ty n os l n tl e leep cortex layer 9 Sl gl t p ake f act ty partly of spot y d r b t on co espond g to tl e k dne s (2) b) At o e l ou Hea y uptake in l er (3) and bone r arro v 8) pers sts Cons de able uptake also e de t n l e u prarenal gla ds (9) h a l gl t p ake tl a spo t ap pearance n tl e k dne s a tl n r m of act ty as prese l e pe ard al e do l el (10) c) At 24 l ours Mu l act ty ren a ne l in tl e l e 3 d bone ma o 8 Tl e expo ure o l n ng tl e nte nal vall (11) as probably due to l a t y n tl e p e one l endo l el Fa nt a t v correspond g to tl e lungs 4 s na l bular sal y gla d 6 o l rger e el 2 could be traced 1 spleen 2 k dney 3 l e 4 lung 5 l ea t 6 sal y gl l 7 p la e a a d amn on ep tl el um 8 verteb ae 9 suprarenal gla d 10 pe a d al e do l el n 11 per tone 12 aorta v tl ts con e of cula ng bloo l

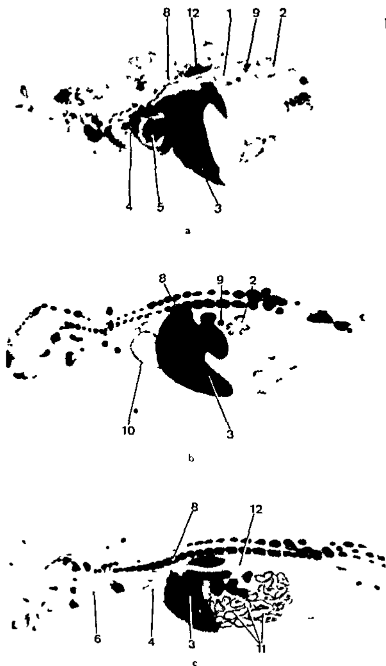


Fig 5 (For legend see opposite page)

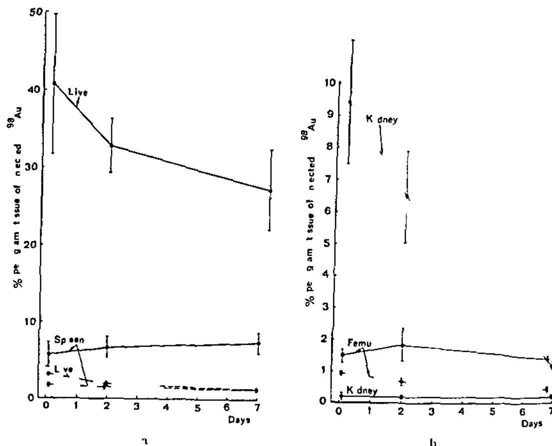


Fig 4 The relat e amo nt of ¹⁹⁸A per gram t ss e in a) l er and splee a d b k d e a d fe n r 2 l o rs 24 l ours and 7 days after intraveno s injecti o s of ¹⁹⁸A sol t on eutral e l gol l cl lor de) ( ) a d ¹⁹⁸A i ollo l (——)

F g 5 A tor d ogram s of three m ce k lled at ar o s t es after the ntra e o s nj t on of collo dal ¹⁹⁸A a At ten n n tes Co s derable act ty j ers s ed n the c cula g blood as ev denced by the l loo l pool n the l eart (5) and larger l loo l e els 12) He y up ake the l ver (3) an l l ngs (4 but o ly sl gl t up ake n l e sp en 1) a l e el e 8) The sup arenal glan ls concen rated act v ty mos ly n l e deep o tex layer 9 Sl gl t p ake of act ty partly of spotty d s r b t on correspond ng to the k lneys (2 b) At one l our Hea y uptake n l er (3) and bone marro v (8) pers sts Cons lerable uptake also e ident n the prarenal glands (9) v th a l gl t ptake l a spotty app earance n the k l eys) a th n r m of act ty as present n the per card al endo l el 10) c) At 4 l ours Much act ty rema ned n the l er 3 an l bo e mar o v 8) The exposure outl n ng the est nal all (11) vas probably due to l e ac y the pe oneal endo l el Fa nt ac ty correspon l ng to the lungs 4) sul m and l ular sal ary gla l b o larger es els 2 could be traced 1 spleen 2 k dney 3 l er 4 lung 5 l ea t 6 al ary g n l 7 p a e a d amn on ep tl el um 8 erteb ae 9 p arenal gla d 1 pe a l al en lo l el u n 11 per toneu 12 aorta v th its con e of ulat ng bloo l

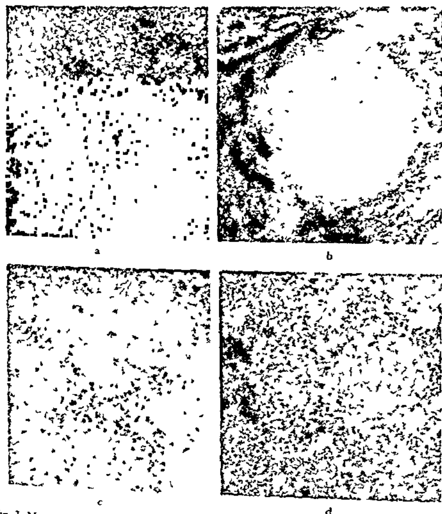
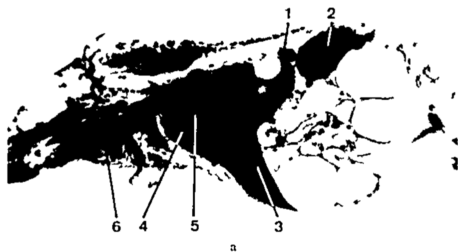


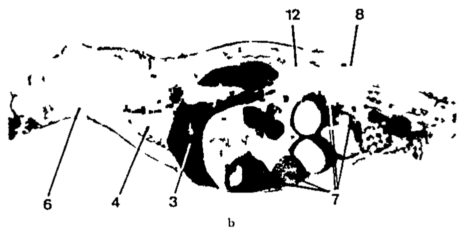
Fig 7 Micro-autoradiogram ... liver and b) d) spleen A ... or solution (c d) the distrib

Fig 6 Autoradiograms of three mice killed at various times after injection of ^{198}Au solution
a) At 10 minutes Blood activity still high ... also

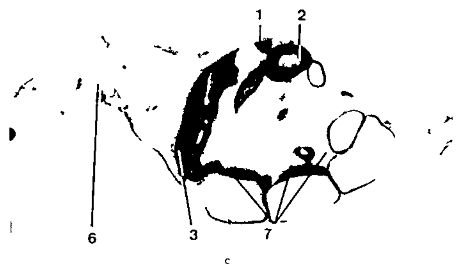
... and ammon-epithelium 8 = vertebrae ... peritoneum 12 – aorta with its content ...



a

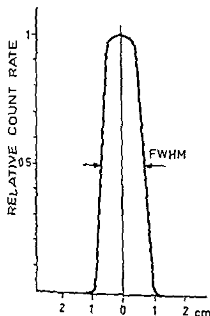


b



c

Fig 6 (For legend see oppos te page)



Point spread function of the collimator in the scanning direction Full width of half maximum = 1 4 cm

Statistical method The non-parametric median test of Westenberg-Mood was used (BRADLEY 1968), the nonirradiated and irradiated mice being compared regarding the sum of counts recorded over the stomach, expressed as percentages of the total number of counts registered over the specimen

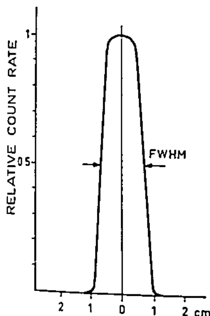
The analysis of the distribution of the radiation activity in the small intestine was performed in the following way When five per cent of the total number of counts recorded over the small intestine had been registered, the detector had passed over a certain length This distance was expressed as a percentage of the total length of the small intestine as the latter was varying from animal to animal The median values of the distances were calculated and compared for each group of irradiated and nonirradiated mice In the same way a second length corresponding to ten per cent, a third length corresponding to fifteen per cent, etc of the total number of counts was determined The groups of animals were compared at each five per cent level from 5 up to 95 per cent of the sum of the counts recorded over the small intestine

The means and the standard deviations were used to describe the length of the small intestine and the haematocrit Analysis of variance was employed to test if any differences existed between the means of the different groups The following levels of significance were used — = $p > 0\,05$ not significant, * = $0\,01 < p \leqslant 0\,05$, ** = $0\,001 \leqslant p < 0\,01$, *** = $0\,001 \geqslant p$

at the level of the front teeth The animal was kept in a vertical position during the injection, manipulation of the mouse and the injection of the dose required about 15 seconds, the animal being conscious when the procedure was over Each mouse was afterwards placed in a separate metabolic cage for 20 minutes during this time the test meal was propelled through the small intestine but never reached the ileocaecal valve The mouse was then decapitated, the abdomen was opened and a ligature was placed round the distal part of the oesophagus pylorus and ileocaecal valve The stomach and the small intestine were removed by dissection and spread out in a groove in a perspex plate 100 cm in length The yellow colour of the test meal which could be seen through the intestinal wall made it possible to control that the meal did not change position during manipulation of the intestine

Measurements The distribution of the isotope in the stomach and the small intestine was examined with a profile scanning method The detector swept over the specimen at 70 mm per minute starting about 50 mm in front of the upper part of the stomach and moving to the ileocaecal valve The distance between the front of the collimator and the perspex plate was 30 mm The scintillation detector was equipped with a slit lead collimator 100 mm long the slit had a rectangular front opening of 10 mm × 40 mm with the long side placed perpendicular to the groove The width of 10 mm was chosen to afford good compromise between sensitivity and spatial resolution (Figure)

The pulses corresponding to 323 keV radiation from ^{51}Cr were selected from the detector in a pulse height analyzer the count rate being recorded on a strip chart recorder As the distance between the specimen and the detector was constant all the parts were seen by the detector under equal geometric conditions The sum of the counts recorded for each 5 mm distance moved by the detector over the specimen was registered to simplify the calculations simultaneously with the rate meter recordings A mean value calculated from 100 sums of counts/5 mm recorded over the groove was subtracted from each one obtained over the specimen The net count value was then used as an expression of the amount of activity in the corresponding 5 mm part of the specimen permitting determination of the gastric emptying and propulsion in the small intestine A light source device attached to the detector head made it possible to relate the number of counts recorded to the different parts of the specimen i e the stomach and the small intestine The syringe with the test meal was placed in the groove before the meal was given to the mouse and the sum of counts recorded with the technique described It was thus possible to check that the animals received an equal amount of activity



Point spread function of the collimator in the scanning direction Full width of half maximum = 1 4 cm

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Table 3

*Comparison of propulsion rate in the small intestine between nonirradiated and irradiated mice by the median test at different percentage levels of the total number of counts recorded over the small intestine. Each level corresponded to a certain distance passed by the detector over the intestine. The median distances were calculated for each group of irradiated mice together with the group of nonirradiated mice. The numbers of mice with distance values lower than the median are given. * — significantly faster propulsion in irradiated than in nonirradiated mice ($p < 0.05$)*

Dose (rad)	Dose/day (rad)	No of mice	No of counts 10 %	40 %	90 %	95 %
			No of mice			
0		59				
500	250	16	11	11	8	8
1 000	250	25	8	9	8	8
2 000	250	27	16	18	15	14
3 000	250	12	4	2*	0*	1*
4 000	250	12	0*	0*	2*	1*
1 000	500	19	13	14	7	5*
2 000	500	18	10	9	3*	5*

Animals irradiated with 250 rad daily up to a total dose of 500, 1 000 and 2 000 rad were compared with nonirradiated animals for intestinal motility. No significant difference in the rate of propulsion was present. The animals irradiated with a total dose of 3 000 and 4 000 rad had a significantly quicker intestinal propulsion than nonirradiated animals when a comparison was made at the 40 to 95 per cent level of the total amount of counts recorded over the small intestine at 3 000 rad and the 10 to 95 per cent level at 4 000 rad. Those animals given 500 rad per day also had a significantly quicker propulsion than the control animals when the median test was made at the 95 per cent level at 1 000 rad and at the 90 to 95 per cent level at 2 000 rad (Table 3).

The haematocrit in irradiated and nonirradiated animals was compared. An analysis of [illegible] from each [illegible] iffer [illegible] animals tha[illegible] received 250 rad a day. They were significantly lower in those that received 500 rad a day with a total dose of 2 000 rad. These changes may be due either to a decrease in the erythrocyte volume or to an increase in the total plasma volume. The decrease in the haematocrit indicated, however, that no haemoconcentration had occurred in the irradiated animals (Table 4).

Table 1

Gastric emptying in nonirradiated and irradiated mice after 250 rad daily. A test meal containing ^{51}Cr was deposited in the stomach three days after irradiation and the mice were killed 20 minutes later. The number of counts recorded over the stomach are expressed as percentages of the total number of counts obtained over the specimen

Dose (rad)	No. of mice	No. of counts recorded over the stomach (%)				Significance of difference from 0 rad
		$\bar{X}$	s	Min	Max	
0	59	20 8	18 3	2 5	91 0	
500	16	25 3	16 7	7 2	70 5	—
1 000	25	19 9	11 0	2 7	50 3	—
2 000	27	22 7	19 4	8 2	71 3	—
3 000	12	20 1	22 1	9 0	28 7	—
4 000	12	31 9	31 8	4 1	72 1	—

Table 2

Gastric emptying in nonirradiated and irradiated mice after 500 rad daily. A test meal containing ^{51}Cr was deposited in the stomach three days after irradiation and the mice were killed 20 minutes later. The number of counts recorded over the stomach are expressed as percentages of the total number of counts obtained over the specimen

Dose (rad)	No. of mice	No. of counts recorded over the stomach (%)				Significance of difference from 0 rad
		$\bar{X}$	s	Min	Max	
0	59	20 8	18 3	2 5	91 0	
1 000	19	43 7	39 2	12 3	83 7	*
2 000	18	57 2	56 0	20 7	93 2	*

Results

The number of counts registered over the stomach expressed as percentages of the total number of counts obtained over the specimen varied widely in the group of nonirradiated mice. These values represent gastric emptying rate. No difference was evident between the stomach values in this group and the different groups of animals irradiated with 250 rad daily, regardless of the total dose. One possible explanation for this observation might be that the gastric function recovered between each irradiation (Table 1). Retardation of gastric emptying was, however, found in animals irradiated with 500 rad daily up to a total dose of 1 000 and 2 000 rad compared with nonirradiated animals (Table 2).

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*Comparison of propulsion rate in the small intestine between nonirradiated and irradiated mice by the median test at different percentage levels of the total number of counts recorded over the small intestine. Each level corresponded to a certain distance passed by the detector over the intestine. The median distances were calculated for each group of irradiated mice together with the group of nonirradiated mice. The numbers of mice with distance values lower than the median are given. * = significantly faster propulsion in irradiated than in nonirradiated mice ($p < 0.05$)*

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4 000	250	12	0*	0*	2*	1*
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2 000	500	18	10	9	3*	5*

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Dose (rad)	No of mice	No of counts recorded over the stomach (%)				Significance of difference from 0 rad
		$\bar{X}$	$\tilde{X}$	Min	Max	
0	59	26 8	18 3	2 5	91 0	
500	16	25 3	16 7	7 2	70 5	—
1 000	25	19 9	11 0	2 7	50 3	—
2 000	27	22 7	19 4	8 2	71 3	—
3 000	12	20 1	22 1	9 0	28 7	—
4 000	12	31 9	31 8	4 1	72 1	—

Table 2

Gastric emptying in nonirradiated and irradiated mice after 500 rad daily A test meal containing ^{51}Cr was deposited in the stomach three days after irradiation and the mice were killed 20 minutes later The number of counts recorded over the stomach are expressed as percentages of the total number of counts obtained over the specimen

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Discussion

Investigations of the effect of irradiation on the gastrointestinal propulsion have produced controversial results, explained by several factors, including variation in sensitivity to ionising radiation of the species (CONARD 1951) Moreover, it is not possible to compare the effect of whole body with abdominal irradiation It is true that irradiation of the abdomen affects the motility of the alimentary canal although not to the same extent as whole body irradiation (SWIFT et coll 1955) Irradiation of the head alone may interfere with the motility of the alimentary canal

HULSE (1966) has noted that gastric emptying is inhibited immediately after the animal has been firmly fixed in position If the animals are kept immobilised during irradiation, changes in the motility of the gastrointestinal tract may be due to factors other than radiation It is therefore advisable not to begin examination of the animals until a few hours after irradiation Operation, e g laparotomy, may also inhibit gastric emptying for at least three days (NYLANDER & SVENSSON 1968) If a catheter be placed in the duodenum via a laparotomy opening or if the animal be subjected to total gastrectomy, gastrointestinal motility may be affected Investigations of the effect of radiation on intestinal propulsion may therefore give misleading results

CHMELAR et coll (1969) have stated that in mice exposed to whole body irradiation with a total dose of 600 rad gastric emptying is inhibited and this inhibition is greatest about three days after irradiation It would appear that gastrointestinal motility should therefore not be examined before three days following irradiation

The tissues of the gastrointestinal tract are capable of recovery from radiation induced changes (WIERNIK et coll 1966) Hence, disturbance of motility following irradiation may gradually disappear, correlation exists between the radiation dose and the severity of disorders of gastrointestinal motility (WOODWARD & ROTHERMEL 1956) Irradiation of experimental animals with 500 rad a day for two days with a total dose of 1 000 rad delayed gastric emptying while irradiation with 250 rad a day for 16 days (total dose 4 000 rad) did not This difference may be explanined by the assumption that 250 rad produces only mild tissue changes that may disappear within a day The final total effect will then be so small that even a relatively large total dose will not interfere with the gastric emptying Gastric emptying is quickest initially after a meal and then decreases exponentially with time This functional pattern is not affected by experimental operations (NYLANDER & WIKSTROM 1968) It is possible that irradiation does not affect the emptying pattern of the stomach of the animal Since accelerated propulsion of the small intestine occurred in irradiated animals irrespective of delayed gastric emptying the volume of the test meal that passes

Table 4

Haematocrit in groups of mice irradiated to different total doses

Dose (rad)	Dose/day (rad)	No of mice	Haematocrit $\bar{X}$	SD	Significance of difference from 0 rad
0		59	46 0	3 1	
500	250	16	46 6	2 6	—
1 000	250	25	44 8	3 3	—
2 000	250	27	43 8	3 1	—
3 000	250	12	43 0	1 6	—
4 000	250	12	43 1	2 2	—
1 000	500	19	46 0	1 8	—
2 000	500	18	43 1	1 9	*

Table 5

Length of the small intestine in groups of mice irradiated to different doses

Dose (rad)	Dose/day (rad)	No of mice	Length in cm of small intestine $\bar{X}$	SD	Sigficance of difference from 0 rad
0		59	40 7	3 9	
500	250	16	42 8	5 0	—
1 000	250	25	48 9	4 7	***
2 000	250	27	45 6	3 3	**
3 000	250	12	45 4	3 8	—
4 000	250	12	45 8	6 6	—
1 000	500	19	42 9	4 4	—
2 000	500	18	47 3	4 2	***

The small intestines from irradiated animals and nonirradiated animals were spread out on perspex and compared in length An analysis of variance rejected the hypothesis that all the mean values do not differ from each other at the 1 per mille level In those irradiated with 250 rad a day and a total dose of 1 000 or 2 000 rad the intestine was significantly longer than in nonirradiated animals, this also occurred in animals given 500 rad a day with a total dose of 2 000 rad Groups of animals irradiated with a total dose of 3 000 and 4 000 rad were composed of only 12 mice each, which might explain that no differences were evident The differences may be explained by the assumption that the small intestine stretches more readily in irradiated than in nonirradiated animals (Table 5)

Discussion

Investigations of the effect of irradiation on the gastrointestinal propulsion have produced controversial results, explained by several factors, including variation in sensitivity to ionising radiation of the species (CONARD 1951) Moreover, it is not possible to compare the effect of whole body with abdominal irradiation It is true that irradiation of the abdomen affects the motility of the alimentary canal although not to the same extent as whole body irradiation (SWIFT et coll 1955) Irradiation of the head alone may interfere with the motility of the alimentary canal

HULSE (1966) has noted that gastric emptying is inhibited immediately after the animal has been firmly fixed in position If the animals are kept immobilised during irradiation, changes in the motility of the gastrointestinal tract may be due to factors other than radiation It is therefore advisable not to begin examination of the animals until a few hours after irradiation Operation, e g laparotomy, may also inhibit gastric emptying for at least three days (NYLANDER & SVENSSON 1968) If a catheter be placed in the duodenum via a laparotomy opening or if the animal be subjected to total gastrectomy, gastrointestinal motility may be affected Investigations of the effect of radiation on intestinal propulsion may therefore give misleading results

CHMELAR et coll (1969) have stated that in mice exposed to whole body irradiation with a total dose of 600 rad gastric emptying is inhibited and this inhibition is greatest about three days after irradiation It would appear that gastrointestinal motility should therefore not be examined before three days following irradiation

The tissues of the gastrointestinal tract are capable of recovery from radiation induced changes (WIERNIK et coll 1966) Hence, disturbance of motility following irradiation may gradually disappear, correlation exists between the radiation dose and the severity of disorders of gastrointestinal motility (WOODWARD & ROTHERMEL 1956) Irradiation of experimental animals with 500 rad a day for two days with a total dose of 1 000 rad delayed gastric emptying while irradiation with 250 rad a day for 16 days (total dose 4 000 rad) did not This difference may be explanined by the assumption that 250 rad produces only mild tissue changes that may disappear within a day The final total effect will then be so small that even a relatively large total dose will not interfere with the gastric emptying Gastric emptying is quickest initially after a meal and then decreases exponentially with time This functional pattern is not affected by experimental operations (NYLANDER & WIKSTROM 1968) It is possible that irradiation does not affect the emptying pattern of the stomach of the animal Since accelerated propulsion of the small intestine occurred in irradiated animals irrespective of delayed gastric emptying the volume of the test meal that passes

into the small intestine per unit of time may be ignored in the evaluation of the effect of radiation on propulsion.

WETTERFORS et coll. (1965), who used rabbits, considered that whole body irradiation with lethal doses had no obvious effect on the plasma volumes until a few hours before the animals succumbed to the radiation reaction, when the plasma volumes tended to increase. The red cell volume in these animals had undergone a clear reduction. This means lower haematocrits in irradiated animals than in nonirradiated animals. If only a part of the body be irradiated, the effect on the red blood cell volume will probably be less than if the whole body be exposed to irradiation.

In those animals in the present investigation given a total dose of 1 000 rad with 250 rad per day and 2 000 rad, irrespective of the fractionation pattern, the length of the small intestine was significantly greater post mortem than in the nonirradiated animals. The morphologic changes in the mucosa of the small intestine after irradiation are well known (BOND 1963). The mitotic activity in the crypts of the small intestine is inhibited and the number of epithelial cells covering the villi becomes smaller; this results in a shortening of the villi which gradually become denuded. These changes may explain why the small intestine from irradiated animals stretched more readily. However, no differences between the small intestines of irradiated and nonirradiated mice were evident macroscopically.

The distance the isotope migrates in the small intestine expressed relative to the total length of the small intestine cannot be used as a basis for comparison between animals. In one animal, for example, the major part of the test meal may have passed further down the small intestine than in another, yet a test meal deposited lower in the small intestine may not reach the distal point of the intestine as early as in an animal in which the meal is deposited more orally. In an attempt to avoid the problems created by these phenomena, a non-parametric median test was used in the present investigation.

SUMMARY

A test meal containing a radioactive isotope ($Na_2{}^{51}CrO_4$) was deposited in the stomach and its distribution in the alimentary tract measured post mortem in mice treated by different irradiation schedules. Irradiation with 250 rad daily up to a total dose of 1 000 rad had no demonstrable effect on gastric emptying. A daily dose of 500 rad up to a total dose of 1 000 and 2 000 rad inhibited gastric emptying and accelerated propulsion of the contents of the small intestine.

ZUSAMMENFASSUNG

Eine Testmahlzeit, das ein radioaktives Isotop ($Na_2{}^{51}CrO_4$) enthielt, wurde im Magen deponiert und dessen Verteilung im Magen-Darm-Trakt bei Mäusen, die mit verschiedenen

Bestrahlungschemata behandelt waren postmortal untersucht Bestrahlung mit 250 rad taglich bis zu einer Totaldosis von 4 000 rad hatte keinen nachweisbaren Effekt auf die Entleerung des Magens Eine tagliche Dosis von 500 rad bis zu einer Gesamtdosis von 1 000 und 2 000 rad verzogerte die Entleerung des Magens und beschleunigte den Durchtritt des Inhalts des Dunndarms

RÉSUMÉ

Un repas d epreuve contenant un isotope radioactif ($Na_2^{51}CrO_4$) a été injecte dans l estomac de souris et sa distribution dans le tube digestif a ete mesurée post mortem apres traitement par differents schemas d irradiation L irradiation quotidienne par 250 rad jusqu a une dose totale de 4 000 rad n'a pas d effet mesurable sur l evacuation gastrique Une dose quotidienne de 500 rad jusqu à une dose totale de 1 000 et 2 000 rad inhibe l evacuation gastrique et accelere la progression du contenu de l intestin grele

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EXPERIMENTAL PARTICLE RADIATION THERAPY IN ANIMAL NEOPLASIA

I Electron versus roentgen irradiation

S W LIPPINCOTT, J L MONTOUR, J D WILSON, S COHN and R F FLORA

During the past decade the possibilities of using various charged 'heavy' particles for radiation therapy of human neoplasms has been carefully considered by a few investigators having access to accelerators producing beams of either protons deuterons, alpha particles or negative pi mesons (FALKMER et coll 1959, 1962 FOWLER & PERKINS 1961, LIPPINCOTT et coll 1963 a, b, LAWRENCE & TOBIAS 1965) Some modalities have actually been tried experimentally in animals and in a limited number of human cases The noncharged fast neutron is also under careful investigation (FOWLER et coll 1972) The reasons for comparing the therapeutic efficiency (RBE) of these particles with conventional electromagnetic radiation are based upon the theoretical advantages deriving from their physical characteristics that determine the mode of energy deposition (LET) These desiderata include (FALKMER et coll 1959) relatively low entry dose as compared to peak dose at the site of the neoplasm (FALKMER et coll 1962) Essentially uniform ionization may be obtained in a selected volume by

Submitted for publication 22 January 1973

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Table

Experimental particle radiation therapy in animal neoplasia 1 0 MeV electron versus 250 keV roentgen irradiation Transplanted C_3H/HeJ neoplasms in mice

Dose (rad)	1 0 MeV electron irradiation			250 keV roentgen irradiation		
	No irradiated	No with positive growth after irradiation	Percent with positive growth after irradiation	No irradiated	No with positive growth after irradiation	Percent with positive growth after irradiation
1000	11	11	100 0	—	—	—
1500	12	10	83 33	11	10	90 91
2000	11	5	45 45	10	9	90 0
2500	12	3	25 0	12	7	58 33
3000	11	4	36 36	11	8	72 73
3500	10	1	10 0	9	7	77 78
4000	13	1	7 69	10	6	60 0
4500	12	1	8 33	7	2	28 57
5000				11	3	27 27

tion The model system consists of implanting a one mm cube of a 100 per cent transplantable mammary neoplasm into the thigh of the mouse at a selected depth and then determining with a range of single doses which form of radiation is more effective in completely destroying the neoplasm The fall-off in energy deposition from roentgen irradiation is only about 10 per cent from surface to exit in the mouse leg, whereas by selecting the proper energy for the electrons the depth of energy deposition may be controlled The disadvantage with roentgen irradiation in this situation is that a much larger volume of normal tissue is irradiated as compared to electron irradiation With these data as a background for subsequent investigations, a comparison can be made of the conventional modalities with the therapeutic efficiency of protons, alpha particles, negative pi mesons and probably heavy ions

Methods and Materials Monoenergetic 0 5, 1 0 and 1 3 MeV electrons were used for normal tissue tolerance studies and for irradiation of the implanted neoplasms The doses reported are those on the skin surface The dosimetry systems employed have been described by us before (LIPPINCOTT et coll 1970) A tissue equivalent extrapolation ion chamber was used to measure tissue dose at various depths in tissue equivalent plastic under the same irradiation conditions as for the animal exposures Thermoluminescent dosimeters and an NBS-calibrated 1 cm^3 tissue equivalent ion chamber were used in beam uniformity measurements

utilizing a series of consecutive (Bragg) peaks, and (FOWLER & PERKINS 1961) control of depth dose with little forward or lateral scatter.

Historically, the deuteron (TOBIAS et coll 1957) was the first of the heavy charged particles to be used in radiation therapy in animal neoplasia. This was followed by employment of the proton in both animals and man. From these investigations have come the most significant data thus far obtained (FALKMER et coll 1959, 1962, TOBIAS et coll 1957). The alpha particle has been used but sparingly in in vivo work while for the negative pi meson, as yet, no uncontaminated beam has been available. The slowness of progress in the development of this field has been chiefly due to the limitation of the available beams which were designed principally for experiments in physics and not for biomedical research. Currently, some of the new beam sources are much more readily adaptable to making possible well designed therapeutic trial programs in animal neoplasia which then may serve as suitable models for man. Notable examples are the recently developed monoenergetic alpha particle beam at the Space Radiation Effects Laboratory (in Virginia) and the negative pi meson source under construction at Los Alamos, New Mexico. Many of the previous disadvantages of the earlier accelerators will be overcome with these devices. For example, the greatest advantage of the new alpha particle beam is that the primary beam can be degraded to any energy, then refocused and analyzed before final focusing onto the target. Such a final relatively monoenergetic beam is preferable to those that have been used in previous biomedical experiments in which it was necessary to use an initial simple degraded beam with resultant heavy scatter and contamination by the degrader.

It is well recognized that in any scheme of radiation therapy for malignant neoplasms the maximum number of rad that can be delivered therapeutically to a neoplasm is determined to a great extent by the dose that the normal structures in the path of irradiation can tolerate. Usually the total dose required to effectively destroy a given malignant neoplasm cannot be administered as a single dose from any type of radiation because the normal structures cannot tolerate it. For this reason, various plans have been devised so that the desired total effective dose is obtained by giving fractions at selected intervals over a given period of time. Whereas, roentgen and gamma rays deposit energy in tissue in electron tracks in which the energy absorption events are, on the average, relatively far apart (low LET), in the case of 'heavy' charged particle radiation the absorption events are close together (high LET). High LET radiation is considered more effective against anoxic tumor cells than low LET radiation (BERRY & ANDREWS 1963, RAJU et coll 1972).

The purpose of the present investigation is to compare the therapeutic efficiency in animal neoplasia of low energy electrons with that of roentgen irradia-



Fig 2 Twelve days post irradiation with 1 3 MeV electrons (2 8 mm penetration) Whole leg exposure a) 870 rad b) 1 930 rad c) 4 500 rad d) 6 650 rad

dermis or in the outer thin layer of subcutaneous tissue Occasionally, some tumor cells appeared to be deposited at about the point where the trochar was withdrawn and growth took place there Such neoplasms were outside of the one cm diameter field of irradiation and such animals could not be used Direct inoculation in the mid thigh at a right angle to the skin could not be used because the tumor might be deposited too deeply for the electron treated groups in which the maximum depth of energy deposition was chosen to be 2 8 mm

Results

The tolerance of normal tissues to electron exposure at low energies is of importance in evaluating effective tumoricidal doses In Fig 1 0 5 MeV electrons were used to penetrate 1 2 mm the exposed area being 2 cm × 2 cm The doses were 1 320 5 400 6 500 and 12 500 rad respectively, twenty-eight days post irradiation Greying of hair appeared at the lowest dose while at the next dose

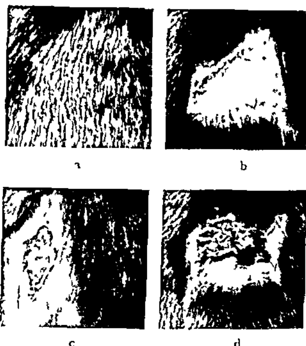


Fig 1 Twenty eight days post irradiation with 0 5 MeV electrons (1 2 mm penetration) a) 1 320 rad b) 5 400 rad c) 6 500 rad d) 12 500 rad

and as reference monitors for the various exposures A Faraday cup receiving the straight ahead electron beam was used to obtain consistency of performance of the electron accelerator

The roentgen ray exposures were obtained using a Westinghouse therapy unit operated at 250 kV and 15 mA with one mm Cu and one mm Al added filtration (First HVL is equal to 2 1 mm Cu) At a distance of 70 cm from the target and under conditions of maximum back scatter the dose-rate was 75 2 R/min as measured with a Baldwin—Farmer dosimeter Various doses were obtained by altering the exposure time The mice were taped to lucite strips with the left hind leg bearing the tumor inoculum extended The animals were then placed, seven at a time, on a turntable that was rotated at 6 rpm under the radiation beam The animals were placed so that the tumor sites were 7 cm from the center of rotation of the turntable and were shielded by a two mm thick piece of lead which contained a one cm wide circular window The lead shield was positioned so that a one cm strip, which included the tumor, was irradiated In this situation, the whole body including the left hind leg beyond the tumor was shielded

The range of doses employed and the number of animals with transplanted neoplasms in each group are shown in the Table The size of the tumor inoculum was about a one mm cube The solid plug of neoplasm was introduced just above the knee joint and extruded from the trochar at the mid point of the thigh in the

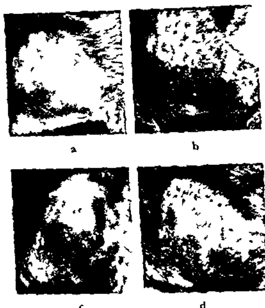


Fig 4 Thirty days post irradiation with 250 keV roentgen a) No irradiation b) 2 000 rad c) 3 000 rad d) 4 000 rad

Before irradiating any neoplasms growth curves were established for non-irradiated transplanted mammary C_3H/HeJ neoplasms in mice (Fig 3) This was essential for several reasons It was necessary to learn how long an animal would live after tumor transplantation and what the rate of tumor growth was This was determined for one mm and for two mm cube tumor transplants and for groups of animals of different sizes to establish how consistent the findings were Composites of the one and two mm cube transplants were then made In doses that did not completely destroy the neoplasm growth curves could then be compared, if desired, with non irradiated tumor growth curves to learn whether a substantial palliative effect occurred Upon a basis of the growth curves (Fig 3) it was decided that by the end of the eleventh week all non irradiated tumor bearing animals were dead and that the findings in tumor irradiated animals should be compared with that time schedule It was also considered that one mm tumor transplants were desirable because with a selected energy of 1 0 MeV for electrons the energy deposition would be limited to 2 8 mm This depth should readily be sufficient for irradiation of the entire neoplasm, while minimizing the amount of normal tissue that would be irradiated

The tumor inoculum was delivered by a device designed to give a uniform volume namely, as close as possible to a one mm cube The implanted neoplasms were allowed to grow for approximately forty-eight hours before irradia-

Fig 3 Growth curves of transplanted mammary C3H/HeJ neoplasms in mice ▲—▲ 2 mm³ implants 25 mice ■—■ 2 mm³ implants 45 mice ●—● 2 mm³ implants 14 mice △—△ 1 mm³ implants 50 mice ○—○ 1 mm implants 16 mice ○—⊙ Composite of 2 mm data □—□ Composite of 1 mm data Numbers indicate animals alive

wet desquamation occurred At the two highest doses deep ulceration resulted, with very slow subsequent healing In Fig 2 1 0 MeV electrons were used to penetrate 2 8 mm, with the whole leg exposed The doses were 870, 1 980, 4 500 and 6 650 rad Twelve days post irradiation no edema was found at the lowest dose It was marked at *the next two doses and excessive at the highest dose* At each of these last three doses ulceration occurred, being maximum at the highest dose With these results in mind, the size of area to be irradiated, depth of penetration, and range of doses were decided upon for the experimental therapeutic schedule

growing as well as a 100 per cent transplantable tumor COHEN & COHEN (1954) presented data on the radiobiology of the C_3H mammary mouse tumor They indicated that for roengten irradiation the LD_{50} for treatment of the neoplasms in situ was 5 700 rad, while for viable fragments irradiated prior to implant 2 000 to 2 500 rad constituted sublethal doses These data helped us to form the range of doses selected for roentgen irradiation in our experiments SUIT et coll (1965) investigated cellular radiation sensitivity of the C3H mammary carcinoma by an analysis of 16 radiation dose response experiments using as the endpoint the permanent local control of tumor All irradiations were given as single doses under conditions of local tissue anoxia and with uniform distribution of radiation dose throughout the tumor The D_{37} was 300 to 325 rad These authors considered that the data were consistent with the proposal that the tumor cells responded to radiation randomly and on the basis of individual cellular radiation sensitivity

FOWLER et coll (1972) investigated the effectiveness of fractionation with roentgen rays and neutrons in mice bearing transplanted C_3H neoplasms The rationale for the clinical use of fast neutrons is that the oxygen enhancement ratio (OER) for neutrons is less than for roentgen rays A comparison was made of the TCD_{50} (dose to control 50 per cent of the tumors) with skin reactions produced in the feet of other mice, using roentgen rays or fast neutrons as single doses or as five or nine fractions at three fractions per week Roentgen rays given as five fractions in nine days were as effective as any of the neutron treatments and were the most effective fractionation scheme tested with that system Neutron treatmen was less dependent upon fractionation

Using a transplantable mouse fibrosarcoma LIPPINCOTT et coll (1971) bombarded one mm cubes of tumor with 20 MeV deuterons Radiation of the entire neoplasm with essentially uniform ionization was achieved by delivering multiple successive Bragg peaks This technique was accomplished by placing a rotating filter of variable thickness in the beam The dose range was from 1 000 to 50 000 rad A single exposure of 1 000 rad was sufficient to destroy all neoplastic growth By the twenty-eighth post irradiation day the site of inoculation of the neoplasm destroyed by the deuteron bombardment contained only fibrotic tissue

In the strong A strain of mice TOBIAS et coll (1957) reported the first experiments designed to localize the ionization of deuterons in transplanted mammary neoplasms The ratio of peak ionization to ionization at the point of entry in Lucite which is roughly equivalent to soft tissue was found to be about 4 1 Tumor bearing mice were treated with deuterons passing only through tumor and skin enclosing it With a specific energy transfer of 6 7 × 10 erg/g complete tumor regression occurred in about one half of the animals in agreement with an

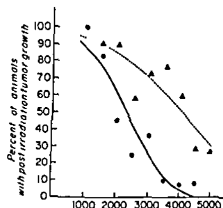


Fig. 5. Dose response curves fitted by probit analysis for electron and roentgen irradiation (dose in rad). ● Electron irradiation. ▲ Roentgen irradiation.

tion. This was to be sure that a viable tumor was growing when irradiation took place. Figure 4 a shows the usual size of growth of the non-irradiated neoplasm at 30 days post-inoculation. In the same figure following 250 keV roentgen irradiation examples of failure to destroy the neoplasm are seen following doses of 2 000, 3 000, and 4 000 rad. At death, all animals were autopsied and sections were taken at the site of irradiation in every exposed animal for histopathologic examination.

The results of irradiating one mm cubes of mammary carcinoma transplanted into the skin of the thigh of C_3H/HeJ mice is shown in the Table. The objective was to determine at similar doses the relative biologic efficiency (RBE) of low energy electrons compared to 250 keV roentgen rays. The endpoint for this was the 50 per cent effect dose (ED_{50}). Dose response curves were fitted to both the electron and roentgen irradiation data by means of a probit analysis (Fig. 5) assuming a normal (Gaussian) tolerance distribution. The chi square test of fit yielded values of the square with six degrees of freedom of 6.85 ($p > 0.25$) and 4.90 ($p > 0.25$) respectively for the electron and roentgen irradiation data, thus indicating that the curves fit the data well. The estimated median effective dose (ED_{50}) for electron treated animals was 2 326 rad with a standard error of 203 and the estimated ED_{50} for roentgen irradiated animals was 4 014 rad with a standard error of 555. Thus, the estimated relative biologic efficiency (RBE) of electron relative to roentgen irradiation therapy is 1.726.

Discussion

The C_3H mammary neoplasm has been used frequently to investigate the effects of various qualities of irradiation upon it. It is a very satisfactory mouse neoplasm with which to work because it is available both as a spontaneously

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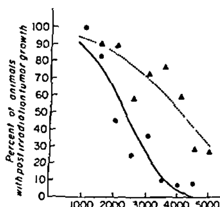


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The C3H mammary neoplasm has been used frequently to investigate the effects of various qualities of irradiation upon it. It is a very satisfactory mouse neoplasm with which to work because it is available both as a spontaneously

growing as well as a 100 per cent transplantable tumor. COHEN & COHEN (1954) presented data on the radiobiology of the C_3H mammary mouse tumor. They indicated that for roengten irradiation the LD_{50} for treatment of the neoplasms in situ was 5 700 rad, while for viable fragments irradiated prior to implant 2 000 to 2 500 rad constituted sublethal doses. These data helped us to form the range of doses selected for roentgen irradiation in our experiments. SUIT et coll. (1965) investigated cellular radiation sensitivity of the C3H mammary carcinoma by an analysis of 16 radiation dose response experiments using as the endpoint the permanent local control of tumor. All irradiations were given as single doses under conditions of local tissue anoxia and with uniform distribution of radiation dose throughout the tumor. The D_{37} was 300 to 325 rad. These authors considered that the data were consistent with the proposal that the tumor cells responded to radiation randomly and on the basis of individual cellular radiation sensitivity.

FOWLER et coll. (1972) investigated the effectiveness of fractionation with roentgen rays and neutrons in mice bearing transplanted C_3H neoplasms. The rationale for the clinical use of fast neutrons is that the oxygen enhancement ratio (OER) for neutrons is less than for roentgen rays. A comparison was made of the TCD_{50} (dose to control 50 per cent of the tumors) with skin reactions produced in the feet of other mice, using roentgen rays or fast neutrons as single doses or as five or nine fractions at three fractions per week. Roentgen rays given as five fractions in nine days were as effective as any of the neutron treatments and were the most effective fractionation scheme tested with that system. Neutron treatmen was less dependent upon fractionation.

Using a transplantable mouse fibrosarcoma LIPPINCOTT et coll. (1971) bombarded one mm cubes of tumor with 20 MeV deuterons. Radiation of the entire neoplasm with essentially uniform ionization was achieved by delivering multiple successive Bragg peaks. This technique was accomplished by placing a rotating filter of variable thickness in the beam. The dose range was from 1 000 to 50 000 rad. A single exposure of 1 000 rad was sufficient to destroy all neoplastic growth. By the twenty-eighth post irradiation day the site of inoculation of the neoplasm destroyed by the deuteron bombardment contained only fibrotic tissue.

In the strong A strain of mice TOBIAS et coll. (1957) reported the first experiments designed to localize the ionization of deuterons in transplanted mammary neoplasms. The ratio of peak ionization to ionization at the point of entry in Lucite, which is roughly equivalent to soft tissue, was found to be about 4 : 1. Tumor bearing mice were treated with deuterons passing only through tumor and skin enclosing it. With a specific energy transfer of 6.7×10 erg/g complete tumor regression occurred in about one half of the animals, in agreement with an

earlier investigation of LAWRENCE et coll. (1965) for 200 kV roentgen rays. This result gave an estimated RBE of about 1.

In rabbits, FALKMER et coll. (1959) reported results with proton and roentgen radiation of V×2 carcinoma in rabbit ears. The RBE appeared to be about 1. No results have been found reported for proton or alpha particle bombardment in mouse mammary neoplasms. Thus, the above comparison of RBE for deuterons and protons is based on transplanted neoplasms in two different species. The sparsity of recorded experiments in the literature, using heavy particle radiation therapy in animals, forms the real basis for our systematic experiments using electrons and roentgen rays as the choice of types of electromagnetic radiation to compare with protons which are currently available at the Oak Ridge Isochronous Cyclotron and alpha particles now obtainable at the Space Radiation Effects Laboratory Accelerator. It is hoped that when the meson factory is completed at Los Alamos negative pi mesons may be added to this comparative series.

SUMMARY

In a comparison of the therapeutic efficiency of low energy electron versus roentgen irradiation of transplanted mammary neoplasm in mice it was found that the ED$_{50}$ for the former was 2 326 rad (±203) and for the latter 4 014 rad (±555). Probit analysis was used to fit the dose response curves. The estimated relative biologic efficiency (RBE) of electron relative to roentgen irradiation at the 50 per cent effect (ED$_{50}$) was 1 726.

ZUSAMMENFASSUNG

In einem Vergleich zwischen der therapeutischen Effektivität von Elektronen mit niedriger Energie gegenüber Röntgenbestrahlung auf transplantierte Brustneoplasmen bei Mäusen wurde für Elektronen eine ED$_{50}$ von 2 326 rad (±203) und für Röntgenstrahlen von 4 014 rad (±555) gefunden. Eine Wahrscheinlichkeitsanalyse wurde verwendet, um die Dosis-Effektkurven anzupassen. Die berechnete relative biologische Effektivität (RBE) der Elektronen gegenüber Röntgenstrahlung bei einem 50 %igen Effekt (ED$_{50}$) betrug 1 726.

RÉSUMÉ

Une comparaison de l'efficacité thérapeutique des électrons de faible énergie et de l'irradiation Roentgen de cancer du sein transplanté chez des souris a montré que la ED$_{50}$ est de 2 326 rad (±203) pour les électrons et de 4 014 rad (±555) pour les rayons de Roentgen. L'analyse des probits a été utilisée pour ajuster les courbes de réponse de dose. L'efficacité biologique relative estimée (RBE) des électrons par rapport à l'irradiation Roentgen pour l'effet 50 pour cent (ED$_{50}$) a été de 1 726.

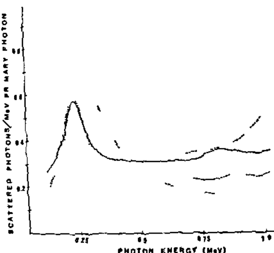


Fig 5 Scattered part of the primary radiation from different ^{60}Co sources — — CORMACK & JOHNS (1958) calculated — — — SCRIMGER & CORMACK (1963) LOFROTH (1970) ——— Present data

Results and Discussion

Stabilatron A typical set of results from a measurement on the Stabilatron ^{60}Co source appears in Fig 4 Good resolution of the two peaks appears at a spreading angle of 15° in the pulse height distribution, the resolution decreases at increasing angles and at 60° no separation of the peaks is apparent The uncertainty of the angle is ± 0 5° By varying the foil thickness between 0 5 and 2 0 mm Al a linear influence on the intensity is obtained with no influence on the resolution this confirms that no important multiple spreading in the foil exists Th...

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5 cm × ... diameter of the collimator in front of the detector is 5 mm The iterated primary photon spectrum (Fig 4) indicates appreciable seperation of the two peaks for angles up to 45°, the ratio between the relative photon content in the two peaks is $I_{1\,1}/I_{1\,33}$ = 0 98 in Fig 4

The scattered radiation in the iterated spectrum coming from scattering within the source itself in the surrounding capsule and from the shield and collimating device is illustrated in Fig 5 It is an average spectrum from five measurements with a spreading angle of 15° SSD = 80 cm and field size 5 cm × 5 cm Comparison with the calculations of CORMACK & JOHNS (1958) and two other measurements (SCRIMGER & CORMACK 1960 LOFROTH 1970) shows excellent agreement with the measurement carried out by LOFROTH with the Siemens Gammatron I with SSD of 1 m and a field size of 5 cm × 5 cm especially for the

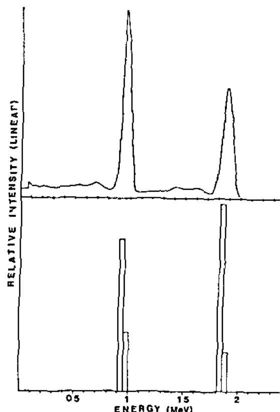


Fig 3 Measured pulse height distribution for the ⁸⁸Y point source (~ 150 μCi) and the iterated photon spectrum after 286 iterations with a 49 × 49 response matrix

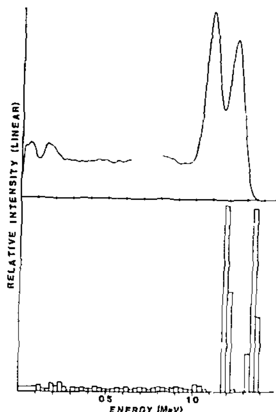


Fig 4 Measured pulse height distribution for the Stabilatron ⁶⁰Co source after 15° spreading in a 0.5 mm Al foil and the iterated primary photon spectrum after 27 iterations with a 99 × 99 response matrix

spacing and bin width proportional to the square root of the energy are employed By placing point sources of known strength in various distances along the collimator axis, the collimator penetration was checked and good agreement with Simons formula for cylindric holes reached Correction was made for the different penetrations at varying energy (SIMONS 1961)

An iterative method was used for unfolding the pulse height distribution (SKARSGÅRD et coll 1961) Fig 3 indicates unfolding of an ⁸⁸Y calibrating spectrum with a 49 × 49 linear matrix The iteration stopped when the convergence ceased or when the criterion by SKARSGÅRD et coll was reached

The computer programmes are written in fortran extended for the CDC—6 400 machine at the University of Århus These analyse the calibration spectra, perform the various corrections and the iterative procedure, and calculate and plot the original primary photon spectrum

Fig 5 Scattered part of the primary radiation from different ^{60}Co sources — —· CORMACK & JOHNS (1958), calculated — — — SCRIMGER & CORMACK (1963) LOFROTH (1970), ——— Present data

Results and Discussion

Stabilatron A typical set of results from a measurement on the Stabilatron ^{60}*Co source* appears in Fig 4 Good resolution of the two peaks appears at a *spreading angle of* 15° *in the* pulse height distribution, the resolution decreases at increasing *angles* and at 60° no separation of the peaks is *apparent* The uncertainty of the angle is $\pm$ 0 5° By varying the foil thickness between 0 5 and 2 0 mm Al a linear influence on the intensity is obtained with no influence *on the resolution*, this confirms that no important multiple spreading in the foil exists The *measurement* has been carried out with SSD (source to skin distance) of 80 cm *for the foil position on* the central axis at which distance the field size is 5 cm × 5 *cm. The diameter* of the collimator in front of the detector is 5 mm The iterated primary photon spectrum (Fig 4) *indicates* appreciable seperation of the two peaks for angles *up to* 45°, *the* ratio between the relative photon content in the two peaks is $I_{1\ 17}/I_{1\ 33}$ = 0 98 in Fig 4

The scattered radiation in the iterated *spectrum coming* from scattering within the source itself in the surrounding capsule and from the *shield and collimating* device is illustrated in Fig 5 It is an average spectrum from five measurements with a spreading angle of 15°, SSD ≈ 80 cm and field size 5 cm × 5 cm Comparison with the calculations of CORMACK & JOHNS (1958) and two other measurements (SCRIMGER & CORMACK 1960, *LÖFROTH 1970*) shows excellent agreement with the measurement carried out by LOFROTH with the Siemens Gammatron I with SSD of 1 m and a field size of 5 *cm* × 5 cm, especially for the

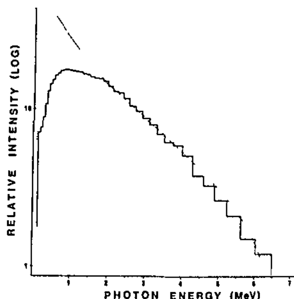


Fig. 6. Experimental bremsstrahlung energy spectrum for the Varian 6 MeV linear accelerator compared with the theoretical thin target curve corrected for filtration in target, window and field flattener filter. ------ Calculated (SCHIFF, 0°), ——— Present data

low energy photons. A difference in the collimating device will produce a deviation in the high energy part of the scattered spectrum according to the work of CORMACK & JOHNS (1958), they calculated that the scattered radiation yields 28.7 per cent of the primary radiation. Integrating their curve up to 1 MeV yields 16.7 per cent, the exposure from the scattered radiation up to 1 MeV formed 13 ± 2 per cent of the primary radiation in the present investigation.

Accelerator. The measurements on the linear accelerator are considerably more difficult due to the pulsating radiation with the enormous photon flux density of 10^9 per cm^2 per pulse at 1 m from the target. The duration of the pulses are 1.5 μs with a frequency of about 100 Hz. On account of the circle time ($\sim$ 100 μs) of the analyser system, it is only possible to obtain at the most one photon for every pulse, which means a relatively long recording time for reasonable statistics. A spectrum measured at 34° ± 0.5° spreading with 1.5 mm Al foil and a 5 mm collimator, FSD = 685 cm, appears in Fig. 6. The dose rate of the accelerator is 200 rad/min, or normal for radiation therapy.

The measurement is compared with a theoretic calculation for a thin target by the SCHIFF formula (1951) integrating all angles of the electrons scattered in the target and with zero degrees for the angle between the incoming electron and the emitted photon (transmission target). A total energy of 7 MeV for the electrons is used in the calculation. The curve is corrected for filtration in the gold target ($1/2\ t_0 = 0.75$ mm), in the nickel window (0.2 mm) and in the field flattener filter of stainless steel (14.2 mm).

Good agreement is secured between the measurement and the thin target curve

for photon energies above 2 MeV Most of the photons radiated from the target in a forward direction come from electrons, which are scattered by small angles, only a small amount of multiple spreading is thus possible (HISDAL 1957)

Since high-energy bremsstrahlung quanta are more likely to be emitted with small angles than low energy ones the spectrum distribution will follow the thin target curve for the high-energy part but deviate for the low-energy part, where the thick target effects are considerable The calculation of the filtration in the target is also somewhat arbitrary and will exert much influence on the low-energy sp"ctrum

Acknowledgements

The author wishes to thank C B Madsen for his interest and encouragement in this work and Prof T Andersen for checking details He also wishes to extend his appreciation to the staff of the laboratory for their help and co-operation The work received the financial sup port of the Danish Medical Research Council

SUMMARY

Measurements have been made of a kilocurie Cobalt 60 unit and a 6 MeV linear ac celerator with a NaI crystal spectrometer Spreading of the primary radiation by Al foil and an iterative procedure for unfolding the pulse height distribution were applied The dose of the scattered part of the primary ^{6} Co radiation reaches 13 $\pm$ 2 per cent of the total The accelerator spectrum is compared with a theoretical thin target curve for bremsstrahlung in a forward direct on

ZUSAMMENFASSUNG

Es wurden Messungen einer Kilokurie Cobolt-60 Einheit und eines 6 MeV Linearaccele rators mit einem NaI Kristall Spektrometer vorgenommen Es wurden die Streuung der Primarstrahlung durch eine Aluminiumfolie und ein Wiederholungsverfahren zur Entfaltung der Pulshohenverteilung verwendet Die Dosis des gestreuten Teils der primaren ^{60}Co-Strahlung erreichte 13 $\pm$ 2 % der Gesamtdos s Das Acceleratorspektrum wird mit einer theoretischen Dunn Target Kurve fur die Bremsstrahlung in Richtung nach vorne verglichen

RÉSUMÉ

L auteur a fait avec un spectromètre à cristal de NaI des mesures sur le rayonnement d un appareil charge d un kilocurie de cobalt 60 et d un accélérateur lineaire de 6 MeV Il a utilise la dispersion du rayonnement primaire par une fe... ...

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